Enterobacter spp.: update on taxonomy, clinical aspect and emerging antimicrobial resistance

Anne P Davin-Regli, Jean Philippe Lavigne, Jean-Marie Pages

Enterobacter spp.: update on taxonomy, clinical aspect and emerging antimicrobial resistance

Anne Davin-Regli *[,1], Jean-Philippe Lavigne [2] and Jean-Marie Pagès [1]

[1] Aix Marseille Univ, INSERM, SSA, IRBA, MCT, Marseille, France.

[2] U1047, INSERM, University Montpellier, Department of Microbiology, University Hospital Nîmes, Nîmes, France

Running title: *Enterobacter* spp. Epidemiology and Resistance

*Corresponding author: Anne Davin-Regli. UMR_MD1, INSERM-1261, Faculté de Pharmacie. 27 Bd Jean Moulin, 13385 Marseille cedex 05, France anne-veronique.regli@univ-amu.fr

TABLE OF CONTENTS

SUMMARY

Enterobacter is a member of the ESKAPE group that contains the major resistant bacterial pathogens. First described in 1960, this group member has proven more complex as a result of the exponential evolution of phenotypic and genotypic methods. Today, twenty-two species belong to the *Enterobacter* genus. These species are described in the environment and have been reported as opportunistic pathogens in plants, animals and humans. The pathogenicity/virulence of this bacterium remains rather unclear due to the limited number of works performed to date in this field. In contrast, its resistance against antibacterial agents has been extensively studied. In the face of antibiotic treatment, it is able to manage different mechanisms of resistance *via* various local and global regulator genes and the modulation of the expression of different proteins, including enzymes (ß-lactamases, etc.) or membrane transporters, such as porins and efflux pumps. During various hospital outbreaks, the *Enterobacter aerogenes* and *E. cloacae* complex exhibited a multidrug resistant phenotype that has stimulated questions about the role of cascade regulation in the emergence of these well-adapted clones.

KEYWORDS *Enterobacter* spp., epidemiology, multidrug resistance, efflux, impermeability, clinical aspects, β-lactamases, pathogenicity, diagnosis

INTRODUCTION

The genus *Enterobacter* includes facultative anaerobic Gram-negative bacilli that are 2 mm long, motile by peritrichous flagella and belong to the family Enterobacteriaceae. It was first described in 1960, but changes in taxonomy have occurred in the last 50 years (1). For example, *E. sakazakii* has been reassigned to a new genus *Cronobacter* (2).

To date, twenty-two species are found in the genus *Enterobacter*: *E. aerogenes, E. amnigenus, E. arachidis, E. asburiae, E. carcinogenus, E. cloacae, E. cowanii, E. dissolvans, E. gergoviae, E. helveticus, E. hormaechei, E. kobei, E. ludwigii, E. mori, E. nimipressuralis, E. oryzae, E. pulveris, E. pyrinus, E. radicincitans, E. soli, E. taylorae* and E. *turicensis*. Among these species, seven are grouped within the *Enterobacter cloacae* complex group: *E. cloacae,* E. *asburiae, E. hormaechei, E. kobei, E. ludwigii, E. mori* and *E. nimipressuralis*. This nomenclature is based on the sharing of phenotypic and especially genotypic characters, obtained by whole genome DNA-DNA hybridizations. Indeed, these five species share at least 60% similarity in their genome with *E. cloacae* (3).

The genus *Enterobacter* is associated with a variety of environmental habitats. These bacteria are recovered from soil and water and are endophytic or considered phytopathogens for various plant species (4). Some species are frequently associated with bioprocessing and metabolic engineering approaches (5, 6). Moreover, *Enterobacter* spp. are also natural commensals of the animal and human gut microbiota. Among these bacteria, only certain subspecies/species have been associated with hospital-acquired infections and outbreaks (7 - 12). *Enterobacter* species are members of ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species) which are described as the leading cause of resistant nosocomial infections (7, 10, 11, 13-20). *Enterobacter aerogenes, E. cloacae* and *E. hormaechei* represent the most frequently isolated species described in clinical infections, especially in immunocompromised patients and those hospitalized in an ICU (Intensive Care Unit), due to

the adaptation of these species to antimicrobial agents and their behavior as opportunistic pathogens. Several hospital outbreaks have been reported in Europe since the mid-1990s, and the wide use of extensive broad-spectrum antibiotics has stimulated the spread of resistant clones (21-23). These pathogens are frequently associated with a multidrug-resistance phenotype, mainly due to their adaptation to the hospital environment and the pathogens' ability to easily acquire numerous genetic mobile elements containing resistance and virulence genes. These species have an intrinsic resistance to ampicillin, amoxicillin, first-generation cephalosporins and cefoxitin due to the expression of a constitutive AmpC β-lactamase. Moreover the production of extended-spectrum β-lactamases has been reported in these bacteria, which make their treatment difficult (24, 25). Antibiotic resistance, regulation of resistant genes and the clinical implications of these situations have been extensively studied (26-31).

The accurate identification of species and subspecies remains a challenge. The development of genome sequencing has rapidly modified the phylogeny of the genus, particularly that of the *E. cloacae* complex (32-34). Due to use of modern molecular techniques, the genus has undergone modifications in classification, and several species have been transferred to and from this genus. For example, four species first identified as *Enterobacter* have been reclassified to the genus *Kosakonia* (*E. cowanii; E. arachidis; E. oryzae and E. radicinintans*), *E. intermedium* has been reclassified to the genus *Kluyvera*, and *E. sakazakii* has been reclassified to the genus *Cronobacter* (2, 35, 36). Moreover, the current taxonomic position of *E. aerogenes* is still discussed. Since 1971, the proposition of reclassification in the genus *Klebsiella* as *K. aerogenes* or *K. mobilis* or *K. aeromobilis* because of its motility remains unverified (37). The phenotypic differences between *E. aerogenes* and the genus *Klebsiella* include motility, the presence of ornithine decarboxylase and the lack of urease activity in *E. aerogenes.* However, results from full genome sequence analysis confirmed that the closest species to *E. aerogenes* was *K. pneumoniae* (37). Considering the taxonomic

requirements, the epithet *mobilis* was illegitimate and *K. aerogenes* was not validated. Thus today *E. aerogenes* is still part of the genus *Enterobacter* and all scientific data use only exceptionally *Klebsiella aerogenes* but again *E. aerogenes* (38). Multilocus Sequence Analysis (MLSA) of housekeeping genes, in part, and sequencing of the 16S rRNA have recently allowed the characterization of new *Enterobacter* species isolated from human infections or from plants (39, 40). The total genome sequence of the various *Enterobacter* has allowed reevaluation of the genus phylogeny and better evaluation of the importance of those incidences where some species were misidentified as other species by routine identification techniques, as was the case of *E. hormaechei* in *E. cloacae* (41).

TAXONOMY OF THE GENUS

Enterobacter amnigenus

The species *E. amnigenus*, described in 1981 by Izard et al., is a rarely isolated bacterium (42). It was suggested by Brady et al. to reclassify it in the genus Lelliotia based on multilocus sequence analysis, however it has never been validly published and *E. amnigenus* still remains the official nomenclature(35).It comprises two genotypically and phenotypically different groups that have been called biogroup 1 and biogroup 2 by the CDC. The strains are generally ODC-positive, LDC- and urease-negative and ferment melibiose. Strains of *E. amnigenus* biogroup 1 ferment sucrose and raffinose but not D-sorbitol. They are ADH-negative. Strains of the *E. amnigenus* biogroup 2 ferment D-sorbitol but not sucrose and raffinose. Additional identifying characters are presented in Table 1. Strains of *E. amnigenus* are generally susceptible to piperacillin, imipenem, gentamicin, tobramycin, amikacin, nalidixic acid, norfloxacin, ciprofloxacin and colistin and are resistant to ampicillin, amoxicillin-clavulanic acid, ticarcillin, and cephalothin. Resistance is variable across strains for second- and third-generation cephalosporins, latamoxef, doxycycline, chloramphenicol and cotrimoxazole (43-45).

Enterobacter cancerogenus

Formerly known as *Erwinia cancerogena*, this species was transferred in 1988 to the genus *Enterobacter* after DNA-DNA hybridizations were studied for the three strains (46). Then, in 1989, Grimont and Ageron observed the synonymy between *E. cancerogenus* and *Enterobacter taylorae* (46). Currently, both denominations are still valid. Nevertheless, the name *E. cancerogenus* should be retained because it was established earlier. These strains are ODC- and ADH-positive and LDC- and urease-negative. They do not ferment D-sorbitol, sucrose, melibiose, dulcitol and raffinose, and they are not gelatinolytic. Additional identification characters are presented in Table 1. Strains studied for *E. cancerogenus* are generally susceptible to third-generation cephalosporins, latamoxef, imipenem, gentamicin, kanamycin, norfloxacin, ciprofloxacin and colistin, and often, they are susceptible to carbenicillin, ticarcillin, azlocillin, mezlocillin and piperacillin. They are naturally resistant to aminopenicillins, some cephalosporins and cotrimoxazole (47). They have an inducible chromosomal AmpC β-lactamase (45, 48-50).

Enterobacter cloacae complex

This complex includes the following species: *Enterobacter asburiae, E. carcinogenus, Enterobacter cloacae, Enterobacter hormaechei, Enterobacter kobei, Enterobacter nimipressuralis* and *E. mori*. All these species are genotypically very close, with more than 60% DNA-DNA homology. A phylogenetic study by Hofmann *et al*., based on sequences of four housekeeping genes, confirmed the genetic diversity of the *Enterobacter cloacae* complex, of which all affiliated species, although genetically related, form distinct clusters (3). *E. cloacae* and *E. hormaechei* are the most frequently isolated in human clinical specimens.

E. asburiae

This species was described in 1986 by Brenner *et al.* (5) from strains of the enteric group 17, of which atypical bacterial strains of the genus *Citrobacter* or of the genus *Enterobacter* had been collected since 1978. It is sometimes described as *Enterobacter muelleri*. These strains are sometimes immobile, often having a Voges-Proskauer (VP)-negative reaction. They are indole-negative, they ferment D-sorbitol and sucrose, and they generally do not ferment the melibiose. When they are VP-positive, it is necessary to differentiate them from *E. cloacae* (*E. asburiae* does not have ADH and does not ferment L-rhamnose) and other VP-positive species (*E. asburiae* does not possess LDC, Tween 80 esterase, or deoxyribonuclease, characters possessed by *Serratia marcescens* and *Serratia liquefaciens*). Additional identifying characters are shown in Table 1. *E. asburiae* has occasionally been described as a bacterium with a clinical significance, mainly in blood cultures. However, de Florio et al. observed its gradual increase in 2017 (51). The complete genome of a clinical *E. asburiae* isolate from a bone marrow transplant patient has been sequenced (52). The strains studied by Brenner et al. (5) were still susceptible to gentamicin and sulfadiazine and generally to carbenicillin, kanamycin, streptomycin, chloramphenicol, nalidixic acid, and colistin. They were all resistant to ampicillin, cefalotine and tetracycline. Environmental strains isolated from watercourses in the United States have been shown as resistant to imipenem by producing a plasmid-derived IMI-2 carbapenemase, thus confirming the hypothesis of an environmental reservoir of this type of resistance gene (53, 54).

E. cloacae

This is the type species of the genus *Enterobacter*. It is now divided into two subspecies: *E. cloacae* subspecies *cloacae,* for which the esculin test is negative, and *E. cloacae* subspecies *dissolvens,* with a positive esculin reaction (55). The strains are ODC- and ADH-positive and LDC-negative (among the bacteria of the genus *Enterobacter,* only the species *E. cloacae, E.*

taylorae, E. kobei and possibly *E. amnigenus* have this profile of decarboxylases). They ferment D-sorbitol, sucrose and melibiose. Additional characters are presented in Table 1. Some pathogenicity factors have been identified as a hemolytic and leukotoxic membrane-cell cytotoxin (56). With regard to epidemiological dissemination, several studies have confirmed that *E. cloacae* colonizations/infections correspond to dissemination of several clusters corresponding to known major multilocus sequences types and that no relationship exists with its geographical source. The various clones have a widespread dissemination and are continuously arising and expanding. Clinical isolates come from various sources and reservoirs, representing sampling from the diversity of the species in the population, and patients are potentially colonized in different ways before entering the hospital (57). *E. cloacae* is naturally resistant to ampicillin, amoxicillin-clavulanic acid, cephalothin and cefoxitin by the low-level production of the Bush Group 1 inducible natural cephalosporinase (class C). Ureidopenicillins and carboxypenicillins are active on at least half of the strains (12). In AmpC chromosomal cephalosporinase, derepression and constitutive production by mutation can lead to resistance to a large number of β-lactams, particularly third-generation cephalosporins, except for cefepime (12, 58, 59). This AmpC-type resistance accounts for 50% of β-lactam resistance in clinical strains and coexists frequently with that due to Extended-Spectrum β-Lactamase (ESBL) expression.

In 1989, the first cases of nosocomial infections due to strains possessing a broad spectrum β-lactamase (ESBL) were identified (18). Since then, various ESBLs, such as TEM, SHV and CTX-M, have been characterized in *E. cloacae,* including inhibitor-resistant TEMs (IRP) (60-63). *E. cloacae,* along with *E. coli* and *K. pneumoniae,* is one of the most common Enterobacteriaceae resistant to third-generation cephalosporins. Nevertheless, in recent years, clinical isolates, resistant through the production of carbapenemases, have been identified (64-67). In particular, in Asia, strains harboring IMP, NDM, GIM, or KPC enzymes have been described (68-70). Lee *et al*. found an imipenem resistance rate of 0.4% in *E. cloacae*

(71). For aminoglycosides, the percentages of resistant strains range from 0 to 51% for gentamicin, and from 0 to 34% for amikacin, whereas ciprofloxacin is active in 64 to 100% of cases (12). A recent study shows that 77% of clinical strains in China are plasmid-positive with determinants of aminoglycoside resistance (70). Regarding quinolones, *Enterobacter cloacae* is one of the *Enterobacteria* with *Escherichia coli* and *Klebsiella pneumoniae* in which the resistance of plasmid origin due to the QnrA protein was initially observed (70, 72-74). These determinants of plasmid resistance to fluoroquinolones are found in more than 60% of the strains (70).

Enterobacter hormaechei

This species has been described by O'Hara et al. in 1989 to designate previously assembled strains in the enteric group 75 (75). These are LDC- and gelatinase-negative strains, generally ODC-, ADH- and urease-positive, and they ferment sucrose and L-rhamnose but not D-sorbitol or melibiose. These characters generally make it possible to differentiate this entity from phenotypically close species. Additional identifying characters are shown in Table 1. Hoffmann *et al.* have subdivided *E. hormaechei* into three subspecies based on the differential biochemical characters D-adonitol, D-arabitol, D-sorbitol, D-melibiose and dulcitol: *E. hormaechei* subspecies *oharae,* which ferments only melibiose; *E. hormaechei* subspecies *hormaechei,* which ferments only dulcitol; and *E. hormaechei* subspecies *steigerwaltii,* which ferments all the mentioned compounds except for dulcitol (76). Now, two supplemental subspecies have been characterized by whole genome comparisons and average nucleotide identity complete genome sequencing: E. *hormaechei* subspecies *xiangfangensis* and *hormaechei* subspecies *hoffmannii* (41).

Most strains, described by O'Hara et al., have been isolated from samples of human origin. These isolates were sensitive or moderately sensitive to azlocillin, mezlocillin, piperacillin, cefotaxime, ceftriaxone, ceftazidime, latamoxef, imipenem, gentamicin, tobramycin, amikacin, chloramphenicol, cotrimoxazole, and trimethoprim (75). Rare strains harbored

ESBL and hyperproduced AmpC cephalosporinase, thereby conferring resistance to third-generation cephalosporins (77). Strains producting carbapenemases have been recently identified (78). Davin-Regli *et al*. reported a nosocomial outbreak involving 21 isolates of *E. hormaechei* subspecies *oharae*, which were resistant to fluoroquinolones, and their isolation had always been preceded by treatment in patients with a fluoroquinolone (17).

Enterobacter kobei

The name *Enterobacter kobei* was proposed by Kosako *et al*., to group strains belonging to the NIH 21 group, previously attached to *E. cloacae* (79). These strains differ from *E. cloacae* by a negative VP-reaction. However, recently, a new VP-positive biotype causing urinary tract infection has been characterized (77). The clinical significance of these strains is often uncertain. However, the species is now also concerned by ESBL production (80).

Enterobacter ludwigii

This new species has been described on the basis of genotypic and phenotypic characters of 16 strains with a clinical origin (81). It is genetically close to *E. hormaechei*, and a Biotype 100 Gallery API system was used to distinguish it from other *Enterobacter* by its ability to use myo-inositol and methyl-D-glucopyranose. The whole-genome sequencing of the type strain was conducted (82). The strains are ADH-, ODC- positive and LDC- and urease negative. All strains are naturally resistant to ampicillin, amoxicillin-clavulanic acid and cefoxitin. Some strains of clinical origin hyperproduced AmpC cephalosporinase but were sensitive to cotrimoxazole, gentamicin, imipenem and ciprofloxacin. Moreover, recently a nosocomial bloodstream infection outbreak in neonates, caused by *Enterobacter ludwigii* coharboring CTX-M-8, SHV-12 and TEM-15, and a clinical isolate responsible of a postoperative bone infection, coharboring NDM-1 and OXA-48 carbapenemases in India were described (83, 84).

Enterobacter mori

This species was first described as a phytopathogenic bacterium (87). It was isolated from diseased mulberry roots. The species could be differentiated from closely related species by the presence of lysine decarboxylase activity and the ability to use D-arabitol. The type strain R18-2T (= CGMCC 1.10322T = LMG 25706T) was sequenced. The 16S rRNA gene sequence and MLSA analysis indicated that *E. mori* is closed related to *E. asburiae* (*muelleri*) (4). Sixty-six genes potentially involved in the secretion system have been identified, explaining the phytopathogenic nature of this species (88). However, recently it has been identified as responsible for human infection in Austria, and the isolates carried an IMI-2 carbapenemase (88).

Enterobacter nimipressuralis

This species was first considered as an *Erwinia* before being reclassified as *Enterobacter* in 1986 by Brenner *et al.* (5, 85) on the basis of DNA-DNA hybridizations. Strains of *E. nimipressuralis* are not pigmented and are generally ADH-positive and urease-negative. They ferment D-sorbitol and melibiose but not sucrose. These characters most often make it possible to differentiate them from species that are phenotypically similar to the genus *Enterobacter*. Additional identifying characters are shown in Table 1. No isolation of human origin has been described except from a pseudobacteremia (86). However, Brenner *et al.* believed that this species could be involved in clinical practice because of the phenotypic proximity of these two species to *E. cloacae* (5)*;* strains of clinical origin could be reported under this latter name. Hoffmann cluster X is *E. nimipressuralis* and it has been suggested to be reclassified as *Lelliottia nimipressuralis* by MLSA (3).

Enterobacter aerogenes

This species occurs phenotypically and genotypically as a mobile *Klebsiella pneumoniae* by its peritrichous, ODC-positive, urease-negative and indole-negative flagella (Table 1). Izard et al. proposed to reclassify *E. aerogenes* as *Klebsiella* under the name *Klebsiella mobilis* (89). Taxonomically justified (by DNA-DNA hybridization), this proposal has not been accepted by medical microbiologists, who maintain the name as *E. aerogenes*. From the genome sequencing performed on a resistant clinical isolate, Diene *et al.*, (37) have recently proposed the shift of the *E. aerogenes* species in the genus *Klebsiella* as *K. aeromobilis*. The successive acquisition of additional genes from genetic mobile elements and other species, which are efficiently integrated and translated, contribute to its notable phenotype (37). Interestingly, several genes involved in the bacterial mobility could have been borrowed from *Serratia* genus and the conjugatif plasmid being also constructed from various transposons or genetic mobile elements (37).

E. aerogenes has been regularly involved in nosocomial infections since 1992, particularly in Western Europe (9, 13, 16, 90-93). In 2012, in France, *E. aerogenes* represented the fifth Enterobacterium responsible for nosocomial infections and the seventh gram-negative bacillus. Its prevalence has fallen sharply since 2000 (21). Although *Enterobacter cloacae* is now the *Enterobacter* sp. most frequently isolated in clinical settings and the species expressing the widest panel of new β-lactamases or carbapenemases, *E. aerogenes* more easily leads to septic shock in infected patients, is associated with higher mortality (39% of patients), and shows greater virulence (94, 95).

Strains of *E. aerogenes* have a broad ability to acquire antibiotic resistance mechanisms (96). They possess a low-level natural AmpC chromosomal cephalosporinase (Bush Group 1) that induces resistance to first-generation cephalosporins. The hyperproduction of chromosomic AmpC in clinical strains leads, after induction by the third-generation cephalosporins or after mutation, to resistance to all β-lactams except cefepime,

cefpirome and carbapenems (97). The plasmidic AmpC cephalosporinase gene (blaCMY-10) gives the same phenotypes (98). In 1993, the first cases of nosocomial infections caused by ESBL-producing strains were observed. In 1998, Pitout *et al*. isolated ESBL-producing strains resistant to gentamicin and cotrimoxazole (99). Various ESBLs were identified as the TEM, SHV and CTX β-lactamases family, but the TEM-24 remains associated with the preferential conjugative plasmid of this species (60, 100-105). In these producer strains, the sensitivity to carbapenems is generally preserved. In parallel, a number of imipenem-resistant clinical strains have been described (61, 94, 96, 100). In these isolates, the lack of antibiotic penetration is mainly associated with a modification of the porin expression: an alteration of the balance Omp35/Omp36 is detected and then followed by a total defect of the porins in the strains collected during the treatment (61, 94, 106-109). Interestingly, an original mechanism of impermeability has been reported with the presence of a mutation in the Omp36 that strongly alters the channel properties (107, 108). Finally, since 2008, carbapenemases of the IPM, NDM or KPC type have been described as responsible for carbapenem resistance (96). Moreover, approximately 40% of multidrug resistant (MDR) clinical strains have active efflux (109). Resistance to quinolones is due to modification of the target or due to plasmidic resistance (*qnrS* or *qepA* encoding an efflux pump) transmitted by other species. Finally, total resistance is not an exceptional phenotype in *E. aerogenes*, since a strain resistant to all antibiotics, including colistin by mutation of *pmrA*, has been isolated and studied (37, 110).

Enterobacter gergoviae

This species was described for the first time by Richard et al. in 1976 using a multidrug-resistant hospital strain isolated in Clermont-Ferrand ("*Gergòvia*" near Clermont-Ferrand, France) (111). Its classification was confirmed in 1980 by Brenner *et al.* after a DNA-DNA hybridization study (5, 112). Recently, a suggestion was made to include this species in the genus *Pluribacter* as *P. gergoviae*, based on the sequence analysis of four genes according to

MLSA (35). The strains are generally LDC- and ODC-positive and gelatinase-negative. They are urease-positive and do not ferment inositol, D-sorbitol and mucate, which differentiate them from *E. aerogenes*. Unlike other *Enterobacter* bacteria, *E. gergoviae* does not grow in potassium cyanide broth. Additional characters are presented in Table 1. *Enterobacter gergoviae* is rarely clinically isolated and has been exceptionally resistant to antibiotics (5, 35, 45, 113). Recently, however, y ESBL type SHV and carbapenemase type (IMP or KPC) producers were described in this species (114). With regard to biocides, due to membrane modifications, esterase production and the modulation of enzymes involved in oxidative detoxification, this species has a natural resistance to the parabens, triclosan, and MIT-CMIT, which are preservatives used in this type of product (115, 116). Such results explain the ability of this species to contaminate cosmetics from a source probably of unknown plant origin (117, 118).

Recent species descriptions

Enterobacter bugandensis, E. timonensis, E. massiliensis, E. chengduensis and *E. sichuanensi and E. roggenkampii* were recently described based on a computational analysis of sequenced *Enterobacter* genomes or MLSA of housekeeping genes (20,40, 41, 119, 120). *E. bugandensis* was responsible for a three-month outbreak of septicemia in a neonatal ward in Tanzania and was also identified from the environment of the International Space station and studied for its MDR phenotype (4, 119). On the basis of whole-genome sequencing, this species was found phylogenetically close to *E. hormaechei*. It is capsule-forming and motile, and its biochemical properties are presented in Table 1. All strains harbored a *bla*CTX-M-15 gene and were resistant to quinolones, tetracycline and sulfamides.

E. timonensis and *E. massiliensis* (characterized in Timone hospital laboratory, Marseille, France) were described on the basis of mass spectrometry and 16S rRNA DNA-DNA hybridization among strains isolated from the gut microflora of patients from Africa (40).

These isolates were not associated with human infections. *Enterobacter chengduensis* and *E. sichuanensis,* respectively, were isolated in China from a human blood sample and from urine and considered a new species due to particular phenotypic characteristics and by phylogenetic analysis using MLSA (4, 120). *E. roggenkampii* (rog.gen.kamp′i.i. N.L. gen. m. *Roggenkamp*, was named in honor of Andreas Roggenkamp, a German bacteriologist who helped understanding of the phylogenetic structure of the *E. cloacae* complex. This creates a novel clade of the *E. cloacae* complex, on the basis of clusters determination within the *E. cloacae* complex using *hsp60* as marker genes (41). *Enterobacter roggenkampii* sp. nov. is the type strain for Hoffmann cluster IV.

EPIDEMIOLOGY AND GLOBAL SPREAD

Environmental sources

Members of the genus *Enterobacter* are environmental organisms and opportunistic pathogens of plants and humans. They are commonly found in water, sewage, soil, plants or animal feces (121). *E. amnigenus* biogroups 1 and 2 have been isolated from drinking water, surface water and from wild soils. *E. asburiae* has been isolated from water, soils, plants, food, hospital environments and healthcare staff equipment, such as probes, catheters, etc. (122). *E. cloacae* has been isolated from food, especially from samples of formula containing plants, raw vegetables and roots, as well as from drinking water (45, 123). Dugleux *et al*., described an outbreak of *E. cloacae* septicemia in a hospital due to the contamination of parenteral nutrition preparations stored in a refrigerator (124). Similarly, outbreaks have been described due to human albumin flasks (125), humidifiers and respiratory therapy equipment (126) and hydrotherapy water in a burn unit (127).

E. gergoviae has been isolated from the environment, from fruits and vegetables, from various sterility controls and in batches of various types of cosmetic products (48, 111, 117,

118). The reservoir of *E. gergoviae* is unknown but could be associated with a plant biotope. Numerous *Enterobacter* species are endophytic bacteria and are present in plant rhizospheres.

Human reservoirs and hospital-acquired infections

E. amnigenus has been isolated from respiratory tract samples, wounds, and stool, as well as from a catheter and a series of blood cultures in a patient undergoing cardiac transplantation (43). *E. cancerogenus* has been found in specimens of cerebrospinal fluid, blood, osteomyelitis, bile, tracheal secretions, and urine, for which its clinical significance has been proven. It has also been isolated from skin specimens, without evidence that *E. cancerogenus* was responsible for the infection (49, 50, 129-131). In 1997, Abbott and Janda, reported 5 cases of *E. cancerogenus* infections as bacteremia in patients with significant injuries or trauma (131). In one of them, *E. cancerogenus* was isolated for 2 months in the liquid drainage.

E. asburiae has been isolated from urine, respiratory samples, blood, stool, wounds, skin, gallbladder (52). *E. cloacae* is present in the normal flora of the human gastrointestinal tract. This species is very often isolated in samples of clinical origin: urines, sputum, and blood culture (132, 133). Currently, the bacterium is found frequently during systematic sampling of neonates who have been colonized early (134). It is involved in 10% of postsurgical peritonitis, 5% of nosocomial sepsis and pneumonias and 4% of urinary tract infections (3). Fata *et al.* reported a fatal case of myositis in a neutropenic patient (135). *E. cloacae* has been implicated in cases of endophthalmitis (136), brain abces (137), meningitides (138), spondilodiscitis (139) and endocarditis (140).

E. hormaechei has been isolated from wounds, sputum and from blood cultures. In 1997, Wenger et al. (19) reported an outbreak in 1993 in an ICU involving 10 premature infants and including 5 cases of bacteremia.

Clinical strains of *E. kobei* have been isolated from various clinical samples: blood, sputum, urine and especially intra-abdominal samples. Recently, a nosocomial bloodstream infection outbreak occurred in a Neonatal ICU in a Venezuelan hospital and was caused by *Enterobacter ludwigii* coharboring three different ESBL (84). *E. aerogenes* is quite frequently isolated in human samples (respiratory, urinary, blood, abcess, gastrointestinal, or cutaneous tissues) or from materials such as ureteric stents. The species is isolated particularly from patient hospitalized in ICUs (15, 22, 23, 110, 141). The spread from patient to patient due to inadequate attention to infection control measures, especially hand-washing, represents the main risk factor (16). Particular infections were described as endocarditis, endophthalmitis and postneurosurgical meningitis (142).

Finally, *E. gergoviae* was isolated from respiratory samples, wounds, blood cultures, stools and urine. Except for its involvement in a nosocomial epidemic of urinary tract infections in France in 1976 and an epidemic of bacteremia in newborns following the contamination of a parenteral glucose solution, sporadic cases are described (143), such as pulmonary pneumopathy (144).

CLINICAL ASPECTS

Pathogenicity

Little is known about the pathogenicity and virulence factors of *Enterobacter* spp due to the paucity of studies in this area. Like other enterobacteria, they possess a flagellum. In addition to facilitating motility, flagella possess several other functions: biofilm formation, protein export, and adhesion (145). The adhesion plays an essential role in the tissue invasion and the beginning of the infection. Hassan *et al*. studied thirty-two isolates of *Enterobacter* sp. obtained from urinary tract infections and identified the well-known *fimH* gene (which encodes for the Type 1 fimbriae) in 40% of the isolates. *Enterobacter* spp. also possesses

different endotoxins (12). Barnes *et al*. observed that *Enterobacter* spp. strains secreted *in vitro* enterotoxins, alpha-hemolysins and cytotoxins similar to Shiga-like toxins II "thiol-activated pore-forming cytotoxins" (146, 147). In Gram-negative bacteria, the type III secretion system (TTSS) is recognized as a pathogenicity factor. One study showed that 27% of *E. cloacae* isolated from clinical infections possessed this factor (10). The *E. cloacae* complex strains may also induce apoptosis of Hep2 cells (148). The acquisition of the plasmid pQC described by Paauw *et al*., containing virulence-encoding (*ter* and *sea* genes) and resistance-encoding (*bla*CTX-M-9, *qnrA1, aadB, aadA2*, *sukk* and *sat*) genes contributes to the virulence and adaptation of the *E. cloacae* clade 1 (10). Additionally, *E. hormaechei* has been reported to be more virulent than other species due to the presence of a HPI that is frequently detected on their chromosome.

The ability of bacteria to assimilate iron through chelators is important for bacterial metabolism and for the establishment of infection. The siderophores encoding genes are generally observed in HPI, especially in *Yersinia* spp. Among these genes, the *irp2* gene has been identified in *Enterobacter* spp. (149). Finally, *E. cloacae* complex strains can harbor curli-encoding genes involving host cell adhesion and invasion. A recent study showed that 78% of the clinical strains studied (n=11) had the *csgBA* operon (which encodes for curli). The authors observed a significant correlation between biofilm formation by these strains and *csgA* gene expression (gene coding for the main subunit of curli, curlin) and *csgD* (coding for an activator of the operon) (150).

Among the genus *Enterobacter*, some differences in pathogenicity could be noted between *E. aerogenes* and *E. cloacae* (151). Azevedo *et al*. reported the presence of virulence-encoding genes in *E. aerogenes* that have been identified in *Klebsiella pneumoniae* (151). For instance, *fimH* and *mrkD* genes encoding adhesins of type 1 and type 3 fimbriae and *ycfM* are detected and they play a key role in the bacterial adhesion and in the biofilm formation, which are

important aspect of bacterial virulence (152). About the iron transport, *kfu*, *entB* and *ybtS* genes that are involved in the production of siderophores are identified in *E. aerogenes* (153). In this regard, it is important to note that Kfu is often detected in hypervirulent *K. pneumoniae* strains and *allS* gene, which is involved in the allantoin metabolism, is closely associated with *K. pneumoniae* isolates detected in liver abscesses. Finally, the virulence of TEM-24-producing *E. aerogenes* was evaluated in the *Caenorhabditis elegans* model (94). A significant reduction of *E. aerogenes* virulence was observed in resistant strains that have modifications of membrane permeability involved in drug resistance. This difference is noticeable even if this species exhibits a moderate virulence in this model although the studied strains harbored the HPI virulence factor-encoding genes. The alteration of outer membrane permeability, *e.g.* lack of porins that are a prominent entry pathway for nutrients, has an important impact on bacterial fitness. The antibiotic pressure promotes the emergence of resistant strains having porin deficiency and LPS modifications that generate a nonphysiological membrane state (29). This causes an unfavorable fitness cost that consequently alters the infection/colonization capability (94).

Characteristics of concerned patients

Enterobacter spp. is involved in nosocomial infections and especially in ICUs where it affects immunocompromised patients, such as neonates, premature infants, diabetes mellitus, burned or multiply traumatized patients, and patients with leukemia or who are undergoing immunosuppressive therapy. Invasive procedures, such as catheterization and intubation, which are frequently found in an ICU, represent a main source of infection (100, 154-157). The patients also harbored numerous comorbidities with a high Charlson score. Among them, diabetes mellitus and its main complications (chronic vascular and renal diseases) represent a risk factor for *Enterobacter* spp. infection (154). Finally, *Enterobacter* spp. preferentially affect patients with a long median duration of hospitalization. This time increases the

digestive carriage, which represents a high risk factor for transmission (158, 159). A persistence of digestive carriage over at least a 5-year period was demonstrated (160).

Frequently, the acquisition of *Enterobater* spp. concerned MDR strains.
Nosocomial acquisition and the median number of antibiotics used represent risk factors for these bacteria (161). An ICU stay >14 days, presence of a tracheostomy, prior central venous catheter use, prior receipt of mechanical ventilation and previous exposure to broad-spectrum antibiotics or any antibiotic during the 30 days before the infection were also associated with acquisition of this MDR (162, 163). Due to the immune context of the patients and the high rate of multidrug resistance, the presence of *Enterobacter* spp. in the bloodstream represents a high risk of mortality (154, 156, 164).

Clinical manifestations

In *Enterobacter* spp., *E. aerogenes* and *E. cloacae* complex have been described in various nosocomial outbreaks that correspond to more than 5% of the bacteremia acquired in the hospital, 5% of pneumonia, 4% of UTI and 10% of postsurgical peritonitis cases (12). E*nterobacter* spp. is involved in numerous infections, including cerebral abscess, pneumonia, meningitis, septicemia and wound, urinary tract (particularly catheter-related UTI) and abdominal cavity/intestinal infections (24, 165). This species is especially described in ICUs, as previously mentioned, and has also been involved in sepsis occuring in neonatology (166, 167). Moreover, *E. hormaechei* has also been identified in intravascular device-related infections, in surgical site infections (such as primarily postoperative in orthopedic trauma or related to devices) or notably after organ transplants (162, 168-173).

Within the *E. cloacae* complex, the most isolated species are *E. hormaechei* (clusters VIII and VI) with 40% to 48% of strains isolated, followed by *E. cloacae* cluster III, with 25% to 42% of strains being isolated (11, 34, 174). In 2012, Kremer and Hoffmann were interested in the types of infections caused by the different species of the group (174). They studied 196

strains, which had been isolated from various samples: blood cultures; catheters; pleural fluids; and respiratory, urogenital, digestive and cutaneous samples. In blood cultures, *E. hormaechei* subsp *oharae* (cluster VI) was significantly more prevalent, whereas *E. asburiae* was not represented at all. On the other hand, *E. asburiae* was more frequently isolated in respiratory samples than from other sites. *E. hormaechei* subsp. *steigerwaltii* (cluster VIII) was overrepresented in skin injury swabs, particularly in burns. No clonality relationship was identified between the strains. Paauw *et al*. studied 156 strains and similarly showed that clade 1 was significantly more involved than clade 2 in infections, suggesting this clade had greater pathogenicity (34). This clade is more common in the nosocomial environment, and its implication in infections could be the result of a better adaptation in this environment than a higher pathogenicity. This hypothesis is supported by the detection of the pQC plasmid in clade 1 but not in clade 2 species (10). Reports of several outbreaks of sepsis in neonatal ICUs in Brazil and the United States have been reported, with *E. hormaechei* being implicated (167). In 2016, Akbari *et al*. studied 50 *Enterobacter* strains isolated from UTIs (7). Twenty-five were part of cluster VI, 9 of cluster III and 6 of cluster VIII. Clusters IV, X, XII and XIII were absent (7). In 2009, the first study on the involvement of *E. cloacae* specifically in infected orthopedic implants was published (11). Fifteen strains (71%) belonged to *E. hormaechei* (5 of cluster VI, 10 of cluster VIII), 2 (9%) to *E. cloac*ae cluster III, and 2 (9%) to *E. ludwigii* (cluster V). *E. cloacae* subsp. *cloacae* and *E. asburiae* were only identified once, and other species were not observed. The authors found a significant difference between the prevalence of cluster III in this type of infection compared to that of cluster III in the other samples. Cluster III was less commonly present in infected orthopedic implants compared to their overall distribution. In addition, in the hip prosthesis samples, only *E. hormaechei* was isolated (9 out of 9). The authors hypothesized that different species would be implicated in different infections. Finally, *E. cloacae* was one of the most prevalent species isolated from

diabetic foot infections using a culturomics approach (175). This result confirms its role in wounds and bone infections.

First-line antibiotics and treatment

As infections due to *Enterobacter* are mainly nosocomial, most isolates present a broad resistance to third generation cephalosporins, penicillins and quinolones due to previous treatment of infected patients located in the same or next hospital ward. Some antibiotics remain effective for treatment: for instance, among the beta-lactams, the fourth-generation cephalosporins and carbapenems are the most attractive options, even if limiting carbapenem use should be encouraged, and the aminoglycosides have a good activity.

The use of third-generation cephalosporins and the monobactams (e.g., aztreonam) represents an important risk of *in vivo* derepression of AmpC β-lactamases, that can be due to a mutation in the repressor, during the treatment inducing high-level resistance to many β-lactam antibiotics. The interest in the concomitant use of aminoglycoside to prevent this type of resistance is mixed (9, 176). The use of fourth-generation cephalosporins (e.g., cefepime and cefpirome) seems to be more effective, mostly due to their activity against AmpC hyperproducing *Enterobacter* strains (177). These molecules present i) an efficient diffusion across the outer membrane porins, 2) a significant stability face to chromosomal β-lactamases, and 3) an enhanced affinity for key penicillin-binding proteins located in the *Enterobacter* periplasm compared to older cephalosporins (178, 179). Many publications have demonstrated their interest (180, 181).

Carbapenems are very efficient against a wide variety of enterobacteria (182). AmpC overproducing *Enterobacter* spp. typically remains susceptible to carbapenems. However, the use of carbapenems could induce a loss of porin production and an impermeability of the bacteria (see the corresponding chapter) (154). This resistance remains rare to date.

Recently, the use of piperacillin-tazobactam combination has been observed as a valuable treatment option for bloodstream infections due to *Enterobacter* spp. (180). Different new antibiotics have been tested against *Enterobacter* spp. The novel siderophore cephalosporin cefiderocol presents excellent results against these bacteria (183). Different combinations between cephalosporins and β-lactamase inhibitors (cefepime/zidebactam, cefepime/tazobactam, ceftolozane/tazobactam, ceftazidime/avibactam, etc.) also present high antibacterial efficacy against these pathogens (184-186). However, their use is not encouraged as a first approach in the aim to limit the emergence of bacteria resistant to these new antibiotics solutions.

Finally, aminoglycosides and particularly amikacin remain active in more than 95% of *Enterobacter* spp. These rates were stable over time period. In *Enterobacter*, the aminoglycoside resistance is usually due to the presence of a plasmid coding for aminoglycoside-modifying enzymes (187).

ANTIMICROBIAL RESISTANCE

Development of antimicrobial resistance

Among *Enterobacter* sp., *E. cloacae* and *E. aerogenes* are mainly affected by development of antimicrobial resistance (14, 188). Regarding *E. aerogenes*, ß-lactam uptake is closely associated with the presence of general porins, such as Omp35 and Omp36, which are homologous to the OmpC and OmpF porins that are the archetypes of the general nonspecific enterobacterial porins (189-192).

Several publications have described a modification of the porin pattern present in antibiotic-resistant isolates: resistant *E. aerogenes* can exhibit a shift in the type of porin expressed (Omp35 to Omp36), a reduction in the production level, or the synthesis of a porin exhibiting mutations in the porin structure that alters channel functions (for reviews see: 29,

190). These interplays of membrane impermeability and enzymatic barriers were been first mentioned in H. Nikaido 's model (193, 194).

Consequently, the clinical isolates collected in the patient body during the antibiotherapy present a serious loss in susceptibility for cephalosporins and carbapenems (195, 196). This alteration of porin profiles is also often reported with a concomitant synthesis of degradative enzymes such as β-lactamases, cephalosporinases, or carbapenemases, which generate a worrying level of ß-lactam resistance (195, 197-199).
Moreover, the dissemination of resistance genes via genetic mobile elements is an important aspect of the antibiotic resistance in Gram-negative bacteria in the ESKAPE group (14, 200).

Molecular mechanisms of resistance

Enzymatic barrier and epidemiology (Table 2)

In most of *Enterobacter* spp., the production of β-lactamases is the prominent mechanism responsible for β-lactam resistance and *E. aerogenes* and *E. cloacae* have a broad ability to modulate these mechanisms of resistance. Importantly, these bacteria are able to produce a low level of a chromosomal AmpC β-lactamase-type cephalosporinase that generates a resistance to first-generation cephalosporins (24, 198). The chromosomally acquired resistance promotes the overproduction of this AmpC cephalosporinase, for instance, during incubation with a subinhibitory concentration of carbapenem (201). Following the inactivation of AmpR or the acquisition of a plasmidic *ampC*, an overproduction of AmpC ß-lactamase contributes to the resistance toward the third-generation cephalosporins (24, 202-204). *E. aerogenes* is also able to integrate a large plasmid (130 kb) that contains the *ampC* gene of chromosomal origin (*bla*CMY -10). In the absence of antibiotic pressure, this genetic transmission can contribute to a systematic

spreading of resistance mechanism (205). This AmpC-related resistance described in 50% of clinical isolates is frequently associated with the expression of ESBL (24).

The first hospital-acquired infections caused by these strains that exhibit resistance to common β-lactams due to the expression of ESBL were reported in 1993 (99). TEM-24 enzyme was associated with *E. aerogenes* clonal dissemination in hospitals in France (8, 23, 103, 104). Other TEM types or CTX-M types (e.g., CTX-M-2) are also often reported, but TEM-24 remains associated with preferential conjugative *Enterobacter* plasmid (10, 24, 73, 100, 101). These enzymes contribute to a global resistance towards all ß-lactams except carbapenems (62). In *Enterobacteriaceae, E. cloacae* is now identified as the third most common bacteria resistant to third-generation cephalosporins with enteric *E. coli* and *K. pneumoniae* (206). Different enzymes (ESBL) belonging to TEM, SHV and CTX -M classes have been characterized in *E. cloacae* and these include also resistant TEM inhibitors or inhibitor-resistant TEM (IRT) enzymes. Notably, some variants have been identified while exhibiting CTX-M production, and others, such as TEM or SHV, have been described from epidemic episodes (24). A transfer of a genomic resistance island is also possible in *Enterobacter* spp. For instance, it has been recently described a variant of AGI1 that belongs to the *Salmonella* genomic island/*Proteus* genomic island/*Acinetobacter* genomic island family, in *E. cloacae* (207). This isolate was resistant to all the antibiotics tested except imipenem and amikacin.

Imipenem is the most effective antibiotic for the treatment of *E. cloacae* infections (24). Carbapenemases that belong to NDM and VIM types are identified in *E. aerogenes* and *E. cloacae*. KPC or class D β-lactamases possessing carbapenemase properties such as the OXA-48 type, are identified in Europe/Asia/America (24, 208, 209). In 2010, the CDC reported the first carriage of NDM-1-producing *E. cloacae* in patients previously treated in India (210, 211). Recently, metallo-β-lactamases that comprizes IMP-type enzymes and NDM-, GIM-, VIM- and serine-carbapenemase-type KPC and FRI, have been characterized (24, 212, 213).

OXA-48-type serine carbapenemase seems to be the most prevalent (208, 214). In *E. cloacae*, an increase in the rate of imipenem resistance has been observed. Moreover, an epidemic survey of *E. cloacae* blood infection reported the presence of metallo-β-lactamase (24). *E. cloacae* is the third *Enterobacteriaceae* for the production of carbapenemase and several strains have been described to simultaneously express two carbapenemases (215). A report mentions that KPC enzyme is the most frequently identified in the SMART global surveillance program from 2008 to 2014 (216). This KPC prevalence is also reported, indicating a limited emergence of NDM-1 (217). In contrast, a longitudinal study (2013-2017) performed in China indicates that an NDM producer is predominant in *E. cloacae* (218). Moreover, the carbapenem resistance in the *E. cloacae* complex had noticeably increased in a recent study of carbapenem susceptibility performed by the US Veterans Health Administration 2006-2015 (219). A recent publication based on genomic epidemiology of carbapenemase-producing *Enterobacter* spp. comprising predominantly *E. xiangfangensis* and *E. hormaechei isolates,* reports that the most common enzyme identified is VIM, followed by NDM, KPC, OXA-48 and IMP (220). Finally, an association of carbapenemase production with a loss of porin expression has been demonstrated in strains resistant to the combination of β-lactamase inhibitors (relactam) plus carbapenems (221).

Regarding the aminoglycoside resistance, aminoglycoside-modifying enzymes are distributed in acetyltransferases (AAC), phosphotransferases (APH), adenylyltransferases (AAD or ANT) and 16S rRNA methyltransferases, such as ArmA and RmtB (70). They are often plasmid-encoded or associated with transposable elements, which facilitate the acquisition of resistance phenotypes (165).

The aminoglycoside-modifying genes, e.g., *aac(3)-IIa*, *aac(6')-Ib*, and *ant(2'')-Ia*, are involved in aminoglycoside resistance affecting at different levels tobramycin, gentamicin, and amikacin. The clinical strains frequently contain more than one enzyme (70, 187, 222). The enzymatic resistance to fluoroquinolones has been characterized as a two-point mutation

allele of *aac*(*6′*)-*Ib* [named *aac*(*6′*)-*Ib-cr*], the aminoglycosides resistance enzymatic determinant, which became able to acetylate ciprofloxacin and norfloxacin (70, 223). The association with the *bla*OXA-1 gene in various genetic mobile elements contributes to a rapid spreading of this new mechanism (24).

Membrane-associated mechanisms

Numerous imipenem-resistant clinical strains have been described, and these present a severe alteration of porin expression associated or not with the overexpression of efflux pumps that occur during antibiotic therapy (24, 29).

Membrane-associated mechanisms of resistance including porin defects and increased levels of efflux pumps are now recognized to strongly participate in the MDR phenotype by controlling the internal concentration of antibiotics (Masi et al., submitted). These "concentration barriers" can also induce the emergence/induction of other mechanisms, such as target mutations, e.g., mutated gyrase or the expression of detoxifying enzymes, including β-lactamases (195). Interestingly, the alteration of LPS also has been described in many resistant isolates (224, 225).

i) Omps, Porins and OM permeability

OmpA was first reported and characterized in 1983, and the Tsx channel involved in nucleoside uptake was reported in 1997 in *E. aerogenes* (226, 227). Today, three general (nonspecific) porins have been identified in *Enterobacter* spp, Omp35, Omp36 and Omp37, and, two additional specific porins, LamB and PhoE, have been identified and exhibit some similarities with *E. coli* OMPs that are largely studied (for review see 189, 190, 191, 228). Importantly, due to their specific trimeric organization in the membrane, these OM proteins need important posttranslational steps that (i) perform the maturation of precursor forms, (ii) correctly address the protein into the OM, and (iii) manage the rate and dynamics of the final trimeric assembly of newly synthesized proteins (229-230). Like the archetypes OmpC and

OmpF, Omp35 and Omp36 are assembled in stable trimers, and each subunit contains a hydrophilic channel presenting a typical β-barrel structure organizing an internal eyelet that constricts the lumen and creates a strong transverse electric field guiding the diffusion of charged molecules. Recently, the 3D structures of *E. aerogenes* and *cloacae* porins have been solved, and the trimeric structures have been published (231). Their structures exhibit a high sequence identity and their channel properties, e.g., conductance and selectivity, which are determined by their planar lipid bilayers, are very similar; for instance, the transversal electric field located in the lumen of the channel is well-conserved in the OmpF group, which is more permeable to anionic compounds (232). Regarding the OmpC group, which includes Omp36, OmpE36, and OmpK36 for *E. aerogenes, E. cloacae* and *K. pneumoniae*, a smaller lumen of the pore, a lower conductance (approximately 3 nS), and a higher cation-selectivity (with a P_{K^+}/P_{Cl^-} to approximately 2.1-2.2) are obtained compared to those obtained from the OmpF orthologs (232). Importantly, the channel is able to promote the antibiotic travel across the OM and ensure accumulation inside the periplasmic space, as demonstrated with purified Omp36 or by using intact cells and labeled antibiotics (233-234). Moreover, immunological and functional comparisons of *E. aerogenes* and *E. coli* porins have reported some conserved and variable features in the antigenic profile and in the reception/translocation functions for bacteriocins (235). This illustrates an adaptive evolution of specific exposed domains when the pore activity is preserved (232) (Table 3).

Similarly, to the *E. coli* porin expression (28, 236, 237), the regulation of these OM general channels is sophisticated in *Enterobacter* spp., and several models have been proposed to integrate not only the Mar and Ram regulation cascades but also the two-component system (TCS) regulator pathways that are directly involved in the sensing, transmission and control of porin transcription-translation-assembly (24, 29, 195). These different means of regulation are involved in the *Enterobacter* response and adaptation to the presence of antibiotics: they

represent the first barrier and the main lever for controlling the penetration flux and accumulation level of antimicrobials (see section 3).

ii) Porins and antibiotic activity

Interestingly, a pioneer study has determined that the porin-deficient phenotype corresponds to approximately 6-7% of the β-lactam resistant *E. aerogenes* isolates collected during a one-year period (105, 238). During the antibiotic treatment of patients, a sequential alteration in the balance between Omp35 and Omp36 has been reported: from the expression of Omp36 and 35 in the carbapenem-susceptible isolate, to the Omp36-producing strain lacking Omp35 and exhibiting intermediate susceptibility, and finally, to a carbapenem-resistant isolate having no porins (94, 239). Furthermore, LamB porin can be expressed in place of Omp35 and Omp36, generating a low susceptibility to β-lactam (240). In some isolates, OmpX, a small OM protein, is involved in the downregulation of porin expression that is associated with a decrease of antibiotic susceptibility (241-243). An *E. cloacae* PhoE porin has been purified and characterized; however, no information has been obtained regarding its role in clinical strains (244). Resistant isolates collected from patients who received antibiotherapy were extensively studied with regard to their level of porin expression and pore activity (107, 108, 201, 240, 244). The loss of porins has been reported in many studies carried out on *E. cloacae* and *E. aerogenes* clinical isolates, and due to space limitations, only a limited part of the published studies are indicated herein (10, 202, 221, 245-250).

Importantly, a key mutation has been identified in a resistant isolate: this mutation, causing a Gly to Asp change, located in the eyelet region of the Omp36 lumen induced a strong modification inside the channel conformation causing alteration of both conductance and selectivity. Consequently, the mutated porin promotes a noticeable resistance to β-lactams but preserves a limited nutrient permeation through the porin (107, 108). This selected "porin strategy" maintains a minimal cost fitness for the bacterial cell associated with a solid

decrease of antibiotic diffusion contributing to the resistance (251). Interestingly, as previously mentioned, the porin loss has an impact on the pathogenicity of *E. aerogenes* isolates, which become less virulent in a *Caenorhabditis elegans* model (94). This adverse effect of resistance mechanisms on *K. pneumoniae* virulence has been extensively analyzed in the case of OmpK36 and OmpK35 in a recent review (252, 253). Moreover, similar observations have been reported for *E. coli* (254) and we may hypothesize that the porin expression is necessary for some important steps involved in the virulence or contribute to the envelope stability during colonization or virulence.

In addition to general porins, TolC, the outer membrane channel involved in the efflux activity and secretion (RND), has been described and characterized in *E. aerogenes* and *E. cloacae* (253-255). In addition, *E. aerogenes* TolC and EefC have been documented and present different channel properties determined by using electrophysiology assays (256).

iii) Efflux pumps and antibiotic activity

Multidrug efflux pumps present on the *Enterobacter* genomes belong to the ABC, MF, SMR, MATE, PACE and RND superfamilies previously described (259, 260); for a classification of membrane transporters, see (261), and the Paulsen site (http://www.membranetransport.org/transportDB2/index.html) (Table 3). Inner membrane transporters correspond to a single-membrane protein located in the inner bacterial membrane, and they function as monomers or as dimers (259). These IM transporters pump out the drugs from the cytoplasm (or the inner leaflet of the IM) to the periplasmic space, such as reported for EmmdR or SugE in *E. cloacae* (262, 263). Interestingly, these IM transporters belonging to the SMR or MATE families can cooperate with the RND family in order to efficiently expel antibacterial compounds outside the bacterial cell (264). In the bacterial envelope, an RND complex, the tripartite efflux system that comprises an inner membrane transporter, a periplasmic adapter protein, and an outer membrane channel, recognizes and translocates the drugs across the OM to the external medium (259, 260, 265). These RND efflux pumps

contribute to the removal of a large number of chemically diverse compounds, such as antibiotics, detergents, biocides, preservatives, etc., that are present in the bacterial volume and can be deleterious for the bacteria (259, 266).

With regard to the AcrAB-TolC pump, the complex has been identified and described in *E. cloacae* and *E. aerogenes* (255, 256, 267). The purification and the biochemical characterization have been performed for TolC and EefC of *E. aerogenes*, and their channel properties have been documented (257, 258). Regarding the involvement of this efflux pump in the resistance of clinical strains, various publications have reported the expression of AcrAB-TolC and its contribution in the reduced susceptibility of the isolates produced (201, 239, 240, 255, 268). Finally, the protein AcrZ, which has been described in the *E. coli* AcrZ-AcrAB-TolC efflux pump, has also been characterized in the *Enterobacter/Klebsiella* genome (269, 270).

Recently, the OqxAB operon has been identified in *E. cloacae* and *E. aerogenes* strains, and this efflux pump contributes to a decreased susceptibility to quinolones in *Enterobacteriaceae* (271). In addition, selective efflux pumps also have been identified and described to play a role in heavy-metal resistance/tolerance in *E. hormachei* and *E. asburiae* isolates (272).

An important point is the relevance and the prevalence of AcrAB-TolC in clinical isolates. A study published in 2008 indicated that the evaluation of efflux activity, measured by using an efflux pump inhibitor (PAβN) in clinical isolates collected within an 8-year period (1995-2003), indicated a noticeable increase of efflux expression during this interval (109). Moreover, this study clearly pinpoints the importance of evaluating the prevalence of membrane barrier, e.g., the impermeability due to porin loss or/and efflux expression, in clinical strains submitted to antibiotherapy treatment, as recently mentioned (258). This aspect is important when taking into account the role of AcrAB-TolC in the *E. aerogenes* susceptibility toward macrolides (273).

A main concern is the correlation existing between efflux activity and intracellular accumulation of antibiotics (211). A series of publications focused on fluoroquinolone accumulation in *E. aerogenes* and *E. coli* strains expressing or not the AcrAB-TolC pump has clearly illustrated the impact of the efflux expression on the accumulation rate inside the bacterial cell (274-277). The expression of the AcrAB-TolC complex is able to manage the internal concentration of antibiotics under the threshold required for triggering the bacterial killing (267, 278). Moreover, by using a microspectrofluorimetry method, the authors report that the fluorescence drug signal varied among the individual bacteria in a uniformly treated population (274, 275). These important data illustrate the heterogeneity of the intrabacterial accumulation of an antibiotic during early incubation times. This heterogeneity may reflect different level of resistant phenotypes co-existing in the isogenic population due to different growth phase or division steps (274, 279). This may pave the way for identifying the bacterial adaptation and persister formation inside a bacterial population submitted to antibiotic stress (274, 275).

In addition, these RND pumps are involved in the bacterial pathogenicity and in the acquisition of additional mechanisms of resistance in *Enterobacteriaceae* (197, 258, 266). Regarding *E. cloacae* and using a mouse model for measuring the competitiveness and virulence of AcrAB-TolC parental or deleted *E. cloacae* strains, the G. Bou's group has clearly demonstrated the involvement of this pump in the bacterial physiology (280).

iv) LPS modification and polymyxin susceptibility

Various alterations of the OM structure are also associated with the LPS modifications in *Enterobacter* spp. clinical isolates that induce some changes in polymyxin susceptibility (110, 281-282). In some cases, the plasmid-mediated colistin resistance *mcr*-1 has been identified in resistant *E. aerogenes* and *E. cloacae* strains (282-285). A study reports that the overall prevalence of colistin-resistant corresponds to 0.67% of the total enterobacterial isolates collected during a four-year period. The colistin resistance was higher in *E. cloacae* (4.2%)

than in *E. coli* and *K. pneumoniae* (0.5% and 0.4% respectively). Although the authors reported that this resistance was not associated with the *mcr* genes, unfortunately, the molecular and genetic characterization of this resistance is missing (286). In a recent study, Guérin *et al*. analyzed a collection of 124 strains of the *E. cloacae* complex and concluded that the PhoP/PhoQ TCS would play a role in colistin-resistance regulation (287). This observation is similar to work performed on *E. aerogenes* clinical isolates collected from patients receiving imipenem (239). In a polymyxin-resistant strain, the authors identified mutations located on *pho*Q and *pmr*B, which are part of the well-described TCS controlling the LPS-modifying enzymes (239). The genome of the colistin-resistant strain identified in 2005 has been sequenced and analyzed, and a mutation in *pmr*A has been identified as the cause of the alteration of LPS biosynthesis, which has been previously observed (37, 110). This type of chromosomal mutation that efficiently alters the OM structure and generates a noticeable decrease of the polymyxin activity may be involved in the emergence of resistant strain devoid of *mcr*-1 and *mcr*-2 (249). Moreover, the dynamic of colistin resistance in *E. cloacae* has been recently reported during selective decontamination of a digestive tract in intensive care units, suggesting a possible clonal transmission (288).

Mutations in antibiotics target

Regarding β-lactam resistance, the target mutation occurs rarely in *Enterobacter* spp. However, the diverse β-lactamases reported today are the result of a series of mutations that have successively appeared in the original β-lactamase genes (289). Moreover, mutations affecting the *ampR* gene strains are described in strains where AmpC cephalosporinase is derepressed (202, 290, 291). In MDR *Enterobacter* isolates, multiple point sequence alterations can be foud in *ampC* but are not in the serine active site or the β-lactam binding site and have no correlation with the resistance phenotype (248). However, in other cases was found amino-acids deletion in the Ω loop of *E. cloacae* AmpC,

associated to enzyme competitiveness and point mutations in strains selected with ceftaroline-avibactam , suspected to affect the activity of the enzyme (292).

About the mutations that contribute to antibiotic resistance, the best characterized are those that affect the quinolone target and, more recently, those involved in polymyxin resistance (see previous section). In *Enterobacter* spp., mutations located into the quinolone resistance-determining regions (QRDRs) of targeted enzymes, e.g. gyrase or topoisomerase, have been described to confer high-level resistance (24, 239, 293-295). This is the common resistance mechanism identified in clinical isolates with recent description of plasmid-mediated quinolone resistance (70, 72, 73, 296, 297). Recent studies in South Africa reported that *qnr* genes were commonly detected in resistant *Enterobacter* isolates collected in a hospital (298, 299). Interestingly, recently the *qnrE1* gene was reported as probably originating from the *Enterobacter* spp. chromosome (300). This "target protective mechanism" confers low-level resistance to first-generation quinolones when present alone (72-74). Importantly, these PMRQ mechanisms exhibit a noticeable spread and have been reported in approximately 60% of clinical isolates due to the presence of various genes coding for ESBLs or AmpC-type β-lactamases on the same plasmid (70, 291, 297).

Lastly, MDR has recently been described in *Enterobacter* isolates (*E. cloacae* and *E. aerogenes*) and in MDR-associated porin alteration, target mutation β-lactamase production, and the efflux overexpression that are accumulated during antibiotic treatment (24, 239). Some mechanisms are intertwined and controlled by regulators in a complex genetic cascade.

Multiple-drug resistance and genetic regulation

Recently, several chemical inducers that are able to modulate the expression of *Enterobacter* membrane transporters, including porins and/or efflux pumps, have been described, e.g.,

salicylate, chloramphenicol, etc. (24, 195, 241, 301). Interestingly, the regulation of porin expression is a fast event, occurring shortly after the addition of chemicals in the culture medium or with the addition of subinhibitory concentrations of antibiotics. During incubation with low imipenem concentrations, an increase in the efflux pump expression that is mediated by the overproduction of MarA has been observed during the incubation (105, 245, 301).

i) MarA, RamA, SoxS, RobA (Figure 1)

Regarding global regulators involved in the control of antibiotic resistance, importantly, the RamA regulator has been characterized in *Enterobacter, Salmonella* and *Klebsiella*, but it has not been detected in *Escherichia* in contrast to the Mar regulon (24, 26).

RamA has been detected in *E. aerogenes* and *cloacae* and has generated a noticeable resistance to various antibiotics (chloramphenicol, tetracycline, tigecycline, fluoroquinolones, trimethoprim, etc.), in conjunction with a decreased expression of Omp35 and an active efflux in *E. aerogenes* (24, 273). RamA seems to be a "super regulator" of the membrane permeability, acting directly or via MarA and controlling the influx and the efflux of antibacterial agents in *Enterobacter* (24, 30). In addition, *rarA* may also contribute to the combined regulation of the RamA-MarA cascade during the emergence of antibiotic resistance (302, 303). RarA that belongs to the AraC-type transcriptional regulator is overproduced when the negative regulator OqxR is inactivated (25, 304). SoxS and Rob can also play a role by sharing some information detected via other signaling systems in *Enterobacter* (25, 280). Regarding the environmental stress, H-NS (histone-like structuring nucleoid protein) modulates the level and balance (e.g. Omp35/Omp36 ratio) of porins in the outer membrane in response to osmotic stress (24, 25).

ii) Other regulators (Figure 1)

With these global regulators, several other partners play a key role in monitoring the expression of porins: OmpX, a small OM protein, and different sRNAi, such as Mic35 and Mic36 (241-243). In addition, several TCS, such as EnvZ-OmpR, and CpxA-CpxR, can

regulate the expression level of porins. In parallel, other TCS, PmrA-PmrB, and PhoQ-PhoR involved in the synthesis of LPS, which is involved in the last step of porin assembly in the OM, may also modulate the porin level in the OM. Importantly, some mutations have been identified in TCS, such as PmrAB or CpxAB, indicating that, under antibiotic treatment, clinical strains are able to select mutations that can modify the membrane permeability in order to acquire low susceptibility against used antibiotics (239).

Several local regulators, repressors such as *acrR* or *rarR*, play a role by controlling the expression of efflux pumps; a more detailed description has recently been published of the regulators involved in the control of drug membrane transporters in *Enterobacter* and *Klebsiella* (25), and an illustration of this complex network is presented in Figure 1. This illustration has been constructed by using the publications on this subject and descriptions of the genes and proteins in the databank.

iii) Inducers and chemical effectors

Some inducers are described as binding directly to the repressor MarR, such as salicylate and tetracycline, thereby impairing the repressor action (25). The transcriptional activators respond to a variety of chemically unrelated compounds, including antibiotics, biocides, carbonyl cyanide m-chlorophenylhydrazone, cyclohexane, salicylate, acetylsalicylate (aspirin), acetaminophen, sodium benzoate, paraquat, and phenolic rings (24). However, the exact mode of action of this induction is not yet elucidated, and further investigation are necessary to understand the targeted step in the regulation cascade (Figure 1).

DIAGNOSIS OF SPECIES

The routine identification of *Enterobacter* spp. has been classically performed by an evaluation of their phenotypic characters by commercialized systems: the Api 20E® gallery or Vitek®2 (bioMérieux). If these systems are adapted to differentiate *E. aerogenes* and *E. cloacae*, they are limited to differentiating the subspecies in the *E. cloacae* complex except for *E. cloacae* and *E. asburiae*.

Mass spectrophotometry, which is increasingly used today, can identify the *E. cloacae* complex but fails to differentiate the species within the group. The two most commonly used devices have failures on this identification. *E. nimipressuralis* is identified correctly when, on the other hand, *E. hormaechei, E. cloacae, E. asburiae, E. kobei* and *E. ludwigii* are not discriminated (305, 306).

Molecular biology techniques are needed to identify the species precisely. Sequencing of 16S rRNA is widely used to identify *Enterobacter* spp. but this technology can not differentiate two closely related species, hence its lack of applicability to the case of the *E. cloacae* complex (40). The technique of microarray-CGH or comparative genomic hybridization is also a powerful identification method, but this method is time-consuming, expensive, and therefore difficult to implement routinely. It showed the existence of two distinct clades, genetically different within the complex. Possibly, this method could be coupled with that of MLSA. The first clade is then divided into two clusters, with the second being more heterogeneous and containing five clusters. Most strains associated with infections belong to the first clade (34). Recently, the MLSA wich employs sequencing of four to seven of housekeeping genes such as *atpD,* fusA, *idC,infB, gyrB, leuS, dnaA,pyrG, rplB, rpoB* and *hsp6*0, appeared to be the most valuable tool to identify of *E. cloacae* complex species and the most recent *Enterobacter* species (4, 119). MLSA results are strongly validated by single nucleotide polymorphism analysis by whole genome sequence (4).By sequencing the *hsp60* gene, Hoffman and Roggenkamp discriminated thirteen clusters (clusters I to XII and cluster XIII, corresponding to an unstable sequence crowd) within the group (3, 55). More recently, Beyrouthy *et al*., using the same technique, confirmed characterisation of the two new subspecies of *E. hormaechei* obtained by complete genome sequencing and helped to complete the *Enterobacter cloacae* complex clusters (41, 210) (Table 4). Twelve clusters were attributed to existing species and more rencent described species as *E. roggenkampi*, *E.*

bugandensis and *E. hormaechei* subsp. *hoffmanii* (Table 4). The name *E. hormaechei* is sometimes given as a generic name for strains belonging to different clusters.

Recently, a German team implemented a combination of two techniques to identify the species within the *E. cloacae* complex: mass spectrometry (MALDI-TOF MS) coupled with real-time PCR (306). Since mass spectrometry does not allow the species to be distinguished from each other, this group requires associations to be established using real-time PCR that amplifies the *dnaJ* gene. This gene, identified by Pham *et al*., encodes for a chaperone protein of the Hsp40 family and is specific for the *E. cloacae* species (307). Precise identification of the species in the *E. cloacae* complex is important in the biological diagnosis of infections because species are differently implicated in human pathology (11). Phenotypic techniques can lead to misidentifications between very different species harboring different virulence potentials, such as *E. hormaechei* and *Cronobacter sakazakii*. However, a species identification error has no impact on antibiotic therapy, as the species of the *E. cloacae* complex have the same antibiotic resistance profiles.

CONCLUSION

Until now, only few virulence factors/genes have been described in *Enterobacter*. In addition, the precise regulations and the molecular and functional properties involved in bacterial adaptation to various environmental stresses/conditions have not yet been precisely determined (10, 56, 147, 308-312).

A main biological behavior reported in the *Enterobacteriaceae* seems to be associated with its ability to evade the activity of a huge collection of antibacterial agents, including antibiotics, disinfectants, biocides, etc. For instance, *Enterobacter* possesses a versatile and sophisticated system for regulating the envelope permeability as characterized above in clinical strains. It must be noted that the RamA operon, which is not present in *Escherichia*

sp., is playing a key role both directly and indirectly *via* the Mar operon: it can control the expression of porins and RND efflux complexes and it can complement or link its proper regulation cascade to other global regulators such as SoxS or Rob. The advantage of this additional global regulator associated with Mar regulon remains to be clarified in *E. aerogenes* and *cloacae* where it can play the role of an enhancer ring or accelerate the bacterial adaptation to environmental stress and contribute to the prevalence of these Gram-negative bacteria in human infectious diseases.

The complete genome sequences of *E. aerogenes* and *E. cloacae* strains have enlightened the presence of resistance genes that contribute to the MDR phenotype, virulence, quorum sensing and the competition against other microorganisms (37, 121, 239, 313-316). Moreover, the whole-genome sequencing of clinical isolates collected during patient antibiotherapy in association with proteomic/functional studies has strongly enlightened the strategies developed by this important Gram-negative pathogen (239, 313). With this regards, the genus-level analysis/comparison of *Enterobacter* spp. versus *K. pneumoniae* indicates high genetic variation (average nucleotide diversity 0.16 compared to 0.02) (317). This difference suggests that these individual species may have different mechanisms and evolutionary pressures that govern them. The regulation cascades involved during colonization, virulence, resistance, support physiological changes that ensure rapid and appropriate responses to environmental stresses and this remarkable adaptability explains the *Enterobacter* presence in the ESKAPE group.

The re-emergence of *Enterobacter* as a worrying resistant pathogen is an important health concern, especially when the scarcity of new antibiotics active against Gram-negative bacteria is considered. Consequently, much effort is necessary to identify/dissect the molecular and genetic events that manage the *Enterobacter* adaptation and to clarify the unknown aspects remaining in the regulation cascade.

ACKNOWLEDGEMENTS

The research leading to the discussions presented here was conducted as part of the TRANSLOCATION consortium and has received support from the Innovative Medicines Initiatives Joint Undertaking under Grant Agreement n°115525, resources which are composed of financial contribution from the European Union's seventh framework program (FP7/2007-2013) and EFPIA companies in kind contribution. This work was also supported by Aix-Marseille Univ., INSERM and the Service de Santé des Armées.

J-P. Lavigne belongs to the FHU InCh (Aviesan, University Montpellier, University Hospitals Montpellier and Nîmes).

ABBREVIATIONS

AAC: aminoside-N-acétyltransférases

APH: aminoside-O-phosphotransferases

AAD: Aminosides adénylyltransférases

ANT: aminoside-O-nucléotidyltransférases

ESBL: Extended Spectrum Beta Lactamase

ESKAPE pathogens: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae,Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species

HPI: High Pathogenicity Island

ICU: Intensive Care Unit

IM: Inner Membrane

LPS: Lipopolysaccharide

MDR: Multi Drug Resistance

MIT-CIT: methylisothiazolinone-chloromethylisothiazolinone

MLSA: Multi Locus Sequence Analysis

OM: Outer Membrane

TCS : Two Component Systems

UTI: Urinary Tract Infections

FIGURES and TABLES

Figure: Schematic representation of the regulation pathways that control the expression of porins and efflux pumps and their interconnection in *Enterobacter* spp.

The multiple regulation cascades that can modulate the outer membrane permeability (porins, LPS) and the expression of major efflux pumps (AcrAB, OqxAB) are summarized.

Blue arrows represent transcriptional activation/repression of different genes such as *acrR*, *ompX*, by the global regulators (e.g. Mar/Ram/Sox). Red arrows symbolize the external stress signals that can activate/repress some gene expression. Black arrows indicate the negative regulation on gene expression (thin lane directly on promoter/operator region). Yellow squares illustrate some unknown regulation that can modulate gene expression on promoter/operator regions (represented by dashed boxes) of key loci. Dashed lines represent some hypothesized regulations.

Table 1. Biochemical characters for the identification of species of the genus

Enterobacter (4, 5, 48, 55, 75, 82, 85, 121).

	Yellow Pigment	LDC	ADH	URE	ESC	Fermentations								
						INO	SOR	SAC	MEL	RAF	AMG	DUL	ADO	ARL
E. amnigenus biogr. 1	-	-	-	-	+	-	-	+	+	+	V	-	-	-
E. amnigenus biogr. 2	-	-	V	-	+	-	+	-	+	-	+	-	-	-
E. cancerogenus	-	-	+	-	+	-	-	-	-	-	-	-	-	-
E. asburiae	-	-	(-)	+/-	+	-	+	+	V	(+)	+	-	-	-
E. cloacae subsp. *cloacae*	-	-	+	-	-	(-)	+	+	+	(+)	(+)	(-)	(-)	(-)
E. cloacae subsp. *dissolvens*	-	-	+	+w	+	V	+	+	+	+	+	-	-	-
E. hormaechei subsp. *hormaechei*	-	-	(+)	(+)	-	-	-	+	-	-	+	+	-	-
E. kobei	-	-	+	+	-	+	+	+	+	+	+	V	-	-
E. ludwigii	-	(-)	+	-	-	+	+	+	+	+	+	-	-	
E. nimipressuralis	-	-	-	-	+	-	+	-	+	-	+	-	-	
E. aerogenes	-	+	-	-	+	+	+	+	+	+	+	-	+	
E. gergoviae	-	(+)	-	+w	+	-	-	+	+	+	-	-	-	+
E. mori	+	+	nd	nd	+	nd	+	nd	+	nd	nd	nd	nd	+
E. bugandensis	-	-	+	-	w	+	+	nd	+	+	nd	+	-	nd

LDC lysine decarboxylase, ADH Arginine dihydrolase, URE urease, INO inositol, SOR sorbitol, SAC saccharose; MEL melibiose, RAF raffinose, AMG α-methyl-D-glucoside, ADO adonitol and ARL D arabitol. + : positive reaction (> 90% of strains); (+) generally positive reaction; +w : reaction weakly positive; V : variable reaction; (-) generally negative reaction - : negative reaction (<90% of strains); +/- : variable depending of the method used; nd : not determined.

TABLE 2 Acquired resistances described in *Enterobacter cloacae* complex bacteria

Antibiotics	Mechanisms of resistance		Genes	Species	References
β-lactams	Enzymatic β-lactamases	Class A	*blaTEM*, *blaSHV*, *blaCTX-M*	*E. cloacae*,	(63, 318)
				E. hormaechei	(319)
			blaVEB, *blaGES*/IBC, *blaKPC*,*blaFRI*	*E. cloacae*	(212, 320)
				E. hormaechei	(321)
			blaNMCA, *blaIMI*	*E. cloacae*,	(322)
				E. asburiae	(54)
		Class B	*blaVIM*, *blaGIM*	*E. cloacae*,	(69, 323, 324)
			blaNDM	*E. ludwigii*	(84)
			blaIMP, *blaNDM*	*E. cloacae*	(211,325)
				E. hormachei	
		Class C	*ampC*	*E. cloacae*,	(290, 297)
				E. asburiae,	(3)
				E. hormaechei,	(215)
				E. kobei	(165)
				E. ludwigii	(165)
				E. nimipressuralis	(165)
		Class D	*blaOXA-48*	*E. cloacae*	(324)
	Impermeability/efflux		*acrAB-tolC*, *ompC*, *ompF*, *ompX*	*E. cloacae*	(25,31,267,242, 243, 326)
Fluoroquinolones	Target mutation		*gyrA*, *gyr B*, *parC*, *parE*	*E. cloacae*	(293)
	Enzymatic (acetyltransferase)		*aac(6´)-Ib-cr*	*E. cloacae*	(150)
	Protective mechanism target		*qnr* (A, B, S, C, D)	*E. cloacae*,	(327)
				E. hormaechei	
	Efflux		*qepA*, *acrAB-tolC*, *oqxAB*, *sugE*, *emmdR*	*E. cloacae*	(262, 263, 267)
Aminoglycosides	Enzymatic (acetyl, phospho, nucleotidyltransferase)		*aac*, *aph*, *ant*	*E. cloacae*	(327)
	Methylase		*armA*, *rmtB*	*E. cloacae*	(328)
Cyclines	Efflux		*acrAB-tolC*	*E. cloacae*	(329)
				E. hormaechei	

TABLE 3

Some examples of the membrane proteins identified in *Enterobacter* spp.

Bacteria	Name/protein	Function	characteristics	Reference
E. cloacae				
	OmpE35	general porin	trimer	(232, 235)
	OmpE36	general porin	trimer	(232, 235)
	OmpE37	quiescent porin	trimer	
	OmpX		monomer	(242)
	OmpA	OM architecture	nd	
	LamB			(330)
	PhoE			(244)
	EmmdR	IM transporter		(262, 263)
	SugE	IM transporter		(262, 263)
	TolC	OM channel	trimer	(256)
	AcrA	adapter	nd	(256)
	AcrB	IM transporter	trimer	(256)
E. aerogenes				
	Omp35	general porin	trimer	(232, 245)
	Omp36	general porin	trimer	(232, 245)
	Omp37	quiescent porin	trimer	Bornet unpublished data
	OmpX	small channel	nd	(241)
	OmpA	OM architecture	monomer	(201, 226)
	LamB	maltoporin	trimer	(240)
	PhoE	phosphoporin	nd	nd
	Tsx	nucleoside uptake	nd	(227)
	TolC	OM channel	trimer	(255)
	AcrA	adapter	nd	(255)

	AcrB	IM transporter	trimer	(255)
	EefC	OM channel	trimer	(331, 332)
	EefA	adapter	nd	(331, 332)
	EefB	IM transporter	trimer	(331, 332)

TABLE 4

Distribution of clusters and species of the *Enterobacter cloacae* Complex (CEC) using the *hsp60* sequencing technique (165, 210).

Cluster	Species
Cluster I	*E. asburiae*
Cluster II	*E. kobei*
Cluster III	*E. hormaechei* subsp. *hoffmannii*
Cluster IV	*E.* r*oggenkampii*
Cluster V	*E. ludwigii*
Cluster VI	*E. hormaechei* subsp. *oharae* and *xiangfangensis*
Cluster VII	*E. hormaechei* subsp. *hormaechei*
Cluster VIII	*E. hormaechei* subsp. *steigerwalti*
Cluster IX	*E. bugandensis*
Cluster X	*E. nimipressuralis*
Cluster XI	*E. cloacae* subsp. *cloacae*
Cluster XII	*E. cloacae* subsp. *dissolvens*
Cluster XIII	*E. cloacae* sequence crowd

REFERENCES

1. Hormaeche E, Edwards P; 1960. A proposed genus *Enterobacter*. Int Bull Bacteriol Nomencl Taxon 10 71–74.

2. Iversen C, Mullane N, McCardell B, Tall BD, Lehner A, Fanning S, Stephan R, Joosten H. 2008. *Cronobacter* gen. nov., a new genus to accommodate the biogroups of *Enterobacter sakazakii*, and proposal of *Cronobacter sakazakii* gen. nov., comb. nov., *Cronobacter malonaticus* sp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov., *Cronobacter* genomospecies 1, and of three subspecies, *Cronobacter dublinensis* subsp. *dublinensis* subsp. nov., *Cronobacter dublinensis* subsp. *lausannensis* subsp. nov. and *Cronobacter dublinensis* subsp. *lactaridi* subsp. nov. Int J Syst Evol Microbiol 58:1442-7. doi: 10.1099/ijs.0.65577-0.

3. Hoffmann H, Roggenkamp A. 2003. Population genetics of the Nomenspecies *Enterobacter cloacae*. Appl Environ Microbiol 69: 5306-5318.

4. Singh NK, Bezdan D, Checinska Sielaff A, Wheeler K, Mason CE, Venkateswaran K. 2018. Multi-drug resistant *Enterobacter bugandensis* species isolated from the International Space Station and comparative genomic analyses with human pathogenic strains. BMC Microbiol. 18:175. doi: 10.1186/s12866-018-1325-2.

5. Brenner DJ, McWorther AC, Kai A, Steigerwalt AG, Farmer JJ. 1986. *Enterobacter asburiae* sp nov, a new species found in clinical specimens, and reassignment of *Erwinia dissolvens* and *Erwinia nimipressuralis* to the genus *Enterobacter* as *Enterobacter dissolvens* comb. nov. and *Enterobacter nimipressuralis* comb. nov. J Clin Microbiol 23:1114-1120.

6. Zhuang L, Zhou S, Yuan Y, Liu T, Wu Z, Cheng J. 2011. Development of *Enterobacter aerogenes* fuel cells: from in situ biohydrogen oxidization to direct electroactive biofilm. Bioresour Technol 102:284-9. doi: 10.1016/j.biortech.2010.06.038. Epub 2010 Jul 2.

7. Akbari M, Bakhshi B, Najar Peerayeh S. 2016. Particular Distribution of *Enterobacter cloacae* Strains Isolated from Urinary Tract Infection within Clonal Complexes. Iran Biomed J.;20:49-55. Epub 2015 Oct 25.

8. Bertrand X, Hocquet D, Boisson K, Siebor E, Plésiat P, Talon D. 2003. Molecular epidemiology of *Enterobacteriaceae* producing extended-spectrum β-lactamase in a French university-affiliated hospital. Int J Antimicrob Agents 22:128-133.

9. Chow JW, Fine MJ, Shlaes DM, Quinn JP, Hooper DC, Johnson MP, Ramphal R. Wagener MM, Miyashiro DK, Yu VL. 1991. *Enterobacter* bacteremia: Clinical features and emergence of antibiotic resistance during therapy. Ann Intern Med 115:585-590.

10. Paauw A, Caspers MP, Leverstein-van Hall MA, Schuren FH, Montijn RC, Verhoef J, Fluit AC. 2009. Identification of resistance and virulence factors in an epidemic *Enterobacter hormaechei* outbreak strain. Microbiology 155:1478-88. doi: 10.1099/mic.0.024828-0.

11. Morand PC, Billoet A, Rottman M, Sivadon-Tardy V, Eyrolle L, Jeanne L, Tazi A, Anract P, Courpied JP, Poyart C, Dumaine V. 2009. Specific distribution within the *Enterobacter cloacae* complex of strains isolated from infected orthopedic implants. J Clin Microbiol. 47:2489-95. doi: 10.1128/JCM.00290-09.

12. Sanders WE, Sanders CC. 1997. *Enterobacter* spp.: pathogens poised to flourish at the turn of the century. Clin Microbiol Rev 10:220-241.

13. Allerberger F, Koeuth T, Lass-Florl C, Dierich MP, Putensen C, Schmutzhard E, Mohsenipour I, Grundmann H, Hartung D, Bauernfeind A, Eberlein E, Lupski JR. 1996. Epidemiology of infections due to multiresistant *Enterobacter aerogenes* in a university hospital. Eur J Clin Microbiol Infect Dis 15:517-521.

14. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J. 2009. Bad bugs, no drugs: No ESKAPE! An update from the infectious diseases Society of America. Clin Infect Dis 48: 1–12.

15. Canton R, Olivier A, Coque TM, del Carmen Valera M, Perez-Diaz JC, Baquero F. 2002. Epidemiology of extended-spectrum β–lactamase producing *Enterobacter* isolates in a Spanish hospital during a 12-years period. J Clin Microbiol 40:1237-1243.

16. Davin-Regli A, Monnet D, Saux P, Bosi C, Charrel RN, Barthelemy A, Bollet C. 1996. Molecular epidemiology of *Enterobacter aerogenes* acquisition: one-year prospective study in two intensive care units. J Clin Microbiol 34:1474-1480.

17. Davin-Regli A, Bosi C, Charrel RN, Ageron E, Papazian L, Grimont PAD, Cremieux A, Bollet C. 1997. A nosocomial outbreak due to *Enterobacter cloacae* strains with the *E. hormachei* genotype in patients treated with fluoroquinolones treatement. J Clin Microbiol 35: 1008-1010.

18. De Champs C, Sauvant MP, Chanal C, Sirot D, Gazuy N, Malhuret R, Baguet JC, Sirot J. 1989. Prospective survey of colonization and infection caused by expanded spectrum-beta-lactamase-producing members of the family *Enterobacteriaceae* in an intensive care unit. J Antimicrob Chemother 27:2887-2890.

19. Wenger PN, Tokars JI, Brennan P, Samel C, Bland L, Miller M, Carson L, Arduino M, Edelstein P, Aguero S, Riddle C, O'hara C, Jarvis W. 1997. An outbreak of *Enterobacter hormachei* infection and colonization in an intensive care nursery.

Clin Inf Dis 24:1243-1244.

20. Wu W, Feng Y, Zong Z. 2018. *Enterobacter sichuanensis* sp. nov., recovered from human urine. Int J Syst Evol Microbiol. 2018 Dec;68(12):3922-3927. doi: 10.1099/ijsem.0.003089.

21. Anastay M, Lagier E, Blanc V, Chardon H. 2013. Epidémiologie des bêtalactamases à spectre étendu (BLSE) chez les entérobactéries dans un hôpital du sud de la France, 1997-2007. Path Biol 61:38-43.

22. Arpin C, Coze C, Rogues AM , Gachie JP , Bebear C, Quentin C. 1996. Epidemiological study of an outbreak due to multidrug-resistant *Enterobacter aerogenes* in a medical intensive care unit. J Clin Microbiol 34:2163-2169.

23. Bosi C, Davin-Regli A, Bornet C, Malléa M, Pagès JM, Bollet C. 1999. Most *Enterobacter aerogenes* strains in France belong to a prevalent clone. J Clin Microbiol 37:2165-2169.

24. Davin-Regli A, Pagès JM. 2015. *Enterobacter aerogenes* and *Enterobacter cloacae*; versatile bacterial pathogens confronting antibiotic treatment. Front Microbiol 6:392. doi: 10.3389/fmicb.2015.00392.

25. Davin-Regli A, Masi M, Bialek S, Nicolas-Chanoine MH, Pagès JM. 2016. Antimicrobial resistance and drug efflux pumps in *Enterobacter* and *Klebsiella*. In Li X-Z, Elkins CA, Zgurskaya HI (eds), Efflux-Mediated Drug Resistance in Bacteria: Mechanisms, Regulation and Clinical Implications. Springer International Publishing Switzerland, Adis Springer Nature. doi: 10.1007/978-3-319-39658-3.

26. Chollet R, Bollet C, Chevalier J, Malléa M, Pagès JM, Davin-Regli A. 2002. *mar* Operon involved in multidrug resistance of *Enterobacter aerogenes*. Antimicrob Agents Chemother 46:1093-1097.

27. Chollet R, Chevalier J, Bryskier A, Pagès JM. 2004. The AcrAB-TolC pump is involved in macrolide resistance but not in telithromycin efflux in *Enterobacter aerogenes* and *Escherichia coli*. Antimicrob Agents Chemother 48:3621-3624.

28. Dam S, Pagès JM, Masi M. 2018. Stress responses, outer membrane permeability control and antimicrobial resistance in *Enterobacteriaceae*. Microbiology 164:260-267. doi: 10.1099/mic.0.000613

29. Masi M, Réfregiers M, Pos KM, Pagès JM. 2017. Mechanisms of envelope permeability and antibiotic influx and efflux in Gram-negative bacteria. Nat Microbiol 2:17001. doi: 10.1038/nmicrobiol.2017.1.

30. Molitor A, James CE, Fanning S, Pagès JM, Davin-Regli A. 2018. Ram locus is a key regulator to trigger multidrug resistance in *Enterobacter aerogenes*. J Med Microbiol 67:148-159. doi: 10.1099/jmm.0.000667. Epub 2018 Jan 3.

31. Pérez A, Poza M, Fernández A, Fernández Mdel C, Mallo S, Merino M, Rumbo-Feal S, Cabral MP, Bou G. 2012. Involvement of the AcrAB-TolC efflux pump in the resistance, fitness, and virulence of *Enterobacter cloacae*. Antimicrob Agents Chemother 56:2084-2090. doi: 10.1128/AAC.05509-11.

32. Izdebski R, Baraniak A, Herda M, Fiett J, Bonten MJ, Carmeli Y, Goossens H, Hryniewicz W, Brun-Buisson C, Gniadkowski M, and on behalf of the MOSAR WP2, WP3 and WP5 study groups. 2015. MLST reveals potentially high-risk international clones of *Enterobacter cloacae*. J Antimicrob Chemother 70:48-56. doi: 10.1093/jac/dku359.

33. Liu WY, Wong CF, Chung KM, Jiang JW, Leung FC. 2013. Comparative genome analysis of *Enterobacter cloacae*. PLoS One 8:e74487. doi: 10.1371/journal.pone.0074487.

34. Paauw A, Caspers MPM, Schuren FHJ, Hall MAL, Delétoile A, Montijn RC, Verhoef J, Fluit AC; 2008. Genomic Diversity within the *Enterobacter cloacae* Complex. *PLOS ONE* 3:e3018

35. Brady C, Cleenwerck I, Venter S, Coutinho T, De Vos P. 2013. Taxonomic evaluation of the genus Enterobacter based on multilocus sequence analysis (MLSA): proposal to reclassify *E. nimipressuralis* and *E. amnigenus* into *Lelliottia* gen. nov. as *Lelliottia nimipressuralis* comb. nov. and *Lelliottia amnigena* comb. nov., respectively, *E. gergoviae* and *E. pyrinus* into *Pluralibacter* gen. nov. as *Pluralibacter gergoviae* comb. nov. and *Pluralibacter pyrinus* comb. nov., respectively, *E. cowanii*, *E. radicincitans*, *E. oryzae* and *E. arachidis* into *Kosakonia gen. nov.* as *Kosakonia cowanii* comb. nov., *Kosakonia radicincitans* comb. nov., *Kosakonia oryzae* comb. nov. and *Kosakonia arachidis* comb. nov., respectively, and *E. turicensis*, *E. helveticus* and *E. pulveris* into *Cronobacter* as *Cronobacter zurichensis nom. nov., Cronobacter helveticus comb. nov. and Cronobacter pulveris comb. nov.*, respectively, *a*nd emended description of the genera *Enterobacter* and *Cronobacter.* Syst Appl Microbiol 36:309-19. doi: 10.1016/j.syapm.2013.03.005.

36. Izard D, Gavini F, Leclerc H. 1980. Polynucleotide sequence relatedness and genome size among *Enterobacter intermedium* sp nov and the species *Enterobacter cloacae* and *Klebsiella pneumoniae*. Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt I Orig Reihe C 1:51-60.

37. Diene SM, Merhej V, Henry M, El Filali A, Roux V, Robert C, Azza S, Gavory F, Barbe V, La Scola B, Raoult D, Rolain JM. 2013. The rhizome of the multidrug-resistant *Enterobacter aerogenes* genome reveals how new "killer bugs" are created because of a sympatric lifestyle. Mol Biol Evol 30:369-383. doi: 10.1093/molbev/mss236.

38. Tindall BJ, Sutton G, Garrity GM. 2017. *Enterobacter aerogenes* Hormaeche and Edwards 1960 (Approved Lists 1980) and *Klebsiella mobilis* Bascomb et al. 1971 (Approved Lists 1980) share the same nomenclatural type (ATCC 13048) on the Approved Lists and are homotypic synonyms, with consequences for the name *Klebsiella mobilis* Bascomb et al. 1971 (Approved Lists 1980). Int J Syst Evol Microbiol. 2017 Feb;67(2):502-504.

39. Hartl R, Kerschner H, Gattringer R, Lepuschitz S, Allerberger F, Sorschag S, Ruppitsch W, Apfalter P. 2018. Whole-Genome analysis of a human *Enterobacter mori* isolate carrying a bla_{IMI-2} carbapenemase in Austria. Microb Drug Resist doi: 10.1089/mdr.2018.0098. [Epub ahead of print]

40. Tidjani Alou M, Cadoret F, Brah S, Diallo A, Sokhna C, Mehrej V, Lagier JC, Fournier PE, Raoult D. 2017. '*Khelaifiella massiliensis*', '*Niameybacter massiliensis*', '*Brachybacterium massiliense*', '*Enterobacter timonensis*', '*Massilibacillus massiliensis*', new bacterial species and genera isolated from the gut microbiota of healthy infants. New Microbes New Infect 19:1-7. doi: 10.1016/j.nmni.2017.02.002.

41. Sutton GG, Brinkac LM, Clarke TH, Fouts DE. 2018. *Enterobacter hormaechei* subsp. *hoffmannii* subsp. nov., *Enterobacter hormaechei* subsp. *xiangfangensis* comb. nov., *Enterobacter roggenkampii* sp. nov., and *Enterobacter muelleri* is a later heterotypic synonym of *Enterobacter asburiae* based on computational analysis of sequenced *Enterobacter* genomes. F1000Res. 7:521. doi: 10.12688/f1000research.14566.1.

42. Izard D, Gavini F, Trinel PA, Leclerc H. 1981. Desoxyribonucleic acid relatedness between *Enterobacter cloacae* and *Enterobacter amnigenus* sp nov. Int J Syst Bacteriol 31:35-42.

43. Bollet C, Elkouby A, Pietri P, De Micco P. 1991. *Enterobacter amnigenus* isolated from a heart transplant recipient. Eur J Clin Microbiol Infect Dis 10:1071-1073.

44. Freney J, Husson MO, Gavini F, Madier S, Martra A, Izard D, Leclerc H, Fleurette J. 1988. Susceptibility to antibiotics and antiseptics of new species of the family *Enterobacteriaceae*. Antimicrob Agents Chemother 32:873-876.

45. Muytjens HL, van der Ros-van de Repe J. 1986. Comparative in vitro susceptibilities of eight *Enterobacter* species, with special reference to *Enterobacter sakazakii*. Antimicrob Agents Chemother. 29:367-70.

46. Grimont PAD, Ageron E. 1989. *Enterobacter cancerogenus* (Urosevic, 1966) Dickey and Zumoff 1988, a senior subjective synonym of *Enterobacter taylorae* Farmer *et al* (1985). Res Microbiol 140:459-465.

47. Stock I, Wiedemann B. 2002. Natural antibiotic susceptibility of *Enterobacter amnigenus, Enterobacter cancerogenus, Enterobacter gergoviae* and *Enterobacter sakasakii*. Clin Microbiol Infect 8:564-578.

48. Farmer JJ III, Fanning GR, Davis BR, O'Hara CM, Riddle C, Hickman-Brenner FW, Asbury MA, Lowery VA III, Brenner DJ. 1985. *Escherichia fergusonii* and *Enterobacter taylorae*, two new species of *Enterobactriaceae* isolated from clinical specimens. J Clin Microbiol 21:77-81.

49. Freney J, Gavini F, Ploton C, Leclerc H, Fleurette J. 1986. Isolement d'une souche d'*Enterobacter taylorae* chez un brûlé. Méd Mal Infect 5:320-321.

50. Westblom TU, Coggins ME. 1987. Osteomyelitis caused by *Enterobacter taylorae*, formerly enteric group 19. J Clin Microbiol 25:2432-3.

51. De Florio L, Riva E, Giona A, Dedej E, Fogolari M, Cella E, Spoto S, Lai A, Zehender G, Ciccozzi M, Angeletti S. 2018. MALDI-TOF MS Identification and clustering applied to *Enterobacter* species in nosocomial setting. Front Microbiol 14;9:1885. doi: 10.3389/fmicb.2018.01885.

52. Zhu B, Wang S, Li O, Hussain A, Hussain A, Shen J, Ibrahim M. 2017. High-quality genome sequence of human pathogen *Enterobacter asburiae* type strain 1497-78^{T}. J Glob Antimicrob Resist 8:104-105. doi: 10.1016/j.jgar.2016.12.003.

53. Aubron C, Poirel L, Ash RJ, Nordmann P. 2005. Carbapenemase-producing *Enterobacteriaceae*, US rivers. Emerg Infect Dis 11:260-264.

54. Rotova V, Papagiannitsis CC, Chudejova K, Medvecky M, Skalova A, Adamkova V, Hrabak J. 2017. First description of the emergence of *Enterobacter asburiae* producing IMI-2 carbapenemase in the Czech Republic. J Glob Antimicrob Resist 11:98-99.

55. Hoffmann H, Stindl S, Ludwig W, Stumpf A, Mehlen A, Heesemann J, Monget D, Schleifer KH, Roggenkamp A. 2005. Reassignment of *Enterobacter dissolvens* to *Enterobacter cloacae* as *E. cloacae* subspecies *dissolvens* comb. nov. and emended description of *Enterobacter asburiae* and *Enterobacter kobei*. Syst Appl Microbiol 28:196-205.

56. Paraje MG, Barnes AI, Albesa I. 2005. An *Enterobacter cloacae* toxin able to generate oxidative stress and to provoke dose-dependent lysis of leukocytes. Int J Med Microbiol 295:109-16.

57. Moradigaravand D, Reuter S, Martin V, Peacock SJ, Parkhill J. 2016. The dissemination of multidrug-resistant *Enterobacter cloacae* throughout the UK and Ireland. Nat Microbiol. 1:16173. doi: 10.1038/nmicrobiol.2016.173.

58. Ito A, Nishikawa T, Ota M, Ito-Horiyama T, Ishibashi N, Sato T, Tsuji M, Yamano Y. 2018. Stability and low induction propensity of cefiderocol against chromosomal AmpC β-lactamases of *Pseudomonas aeruginosa* and *Enterobacter cloacae*. J Antimicrob Chemother 73:3049-3052. doi: 10.1093/jac/dky317.

59. Zaher A, Cimolai N. 1998. ERIC-PCR typing profiles of *Enterobacter cloacae* are stable after development of advanced cephalosporin resistance. Int J Antimicrobial Agents 9:165-167.

60. Arpin C, Labia R, Dubois V, Noury P, Souquet M, Quentin C. 2002. TEM-80, a novel inhibitor-resistant β-lactamase in a clinical isolate of *Enterobacter cloacae*. Antimicrob Agents Chemother 46:1183-1189.

61. Bornet C, Davin-Regli A, Bosi C, Pagès JM, Bollet. 2000. Imipenem resistance of *Enterobacter aerogenes* mediated by outer membrane permeability. J Clin Microbiol 38:1048-1052.

62. Pitout JDD, Moland ES, Sanders CC, Thomson KS, Fitzsimmons SR. 1997. β-lactamases and detection of β-lactam resistance in *Enterobacter* spp. Antimicrob Agents Chemother 41:35-39.

63. Szabo D, Melan MAA, Hujer AM, Bonomo RA, Hujer KM, Bethel CR, Kristof K, Paterson DL. 2005. Molecular analysis of the simultaneous production of two SHV-type extended-spectrum beta-lactamases in a clinical isolate of *Enterobacter cloacae* by using single-nucleotide polymorphism genotyping. Antimicrob Agents Chemother 49:4716-4720.

64. Galani I, Souli M, Chryssouli Z, Orlandou K, Giamarellou H. 2005. Characterization of a new integron containing, bla (VIM-1) and aac(6')-IIc in an *Enterobacter cloacae* clinical isolate from Greece. J Antimicrob Chemother 55:634-638.

65. Jin C, Zhang J, Wang Q, Chen H, Wang X, Zhang Y, Wang H. 2018. Molecular Characterization of Carbapenem-Resistant *Enterobacter cloacae* in 11 Chinese Cities. Front Microbiol 9:1597. doi: 10.3389/fmicb.2018.01597.

66. Nordmann P, Mariotte S, Naas T, Labia R, Nicolas M.H. 1993. Biochemical properties of a carbapenem-hydrolyzing beta-lactamase from *Enterobacter cloacae* and cloning of the gene into *Escherichia coli*. Antimicrob Agents Chemother 37:939-946.

67. Raimondi A, Traverso A, Nikaido H. 1991. Imipenem- and meropenem-resistant mutants of *Enterobacter cloacae* and *Proteus rettgeri* lack porins. Antimicrob Agents Chemother 35:1174-1180.

68. Dai W, Sun S, Yang P, Huang S, Zhang X, Zhang L. 2013. Characterization of carbapenemases, extended spectrum β-lactamases and molecular epidemiology of carbapenem-non-susceptible *Enterobacter cloacae* in a Chinese hospital in

ChongqinG. Infect Genet Evol 14:1-7.

69. Hamprecht A, Poirel L, Gottig S, Seifert H, Kaase M, Nordmann P. 2013. Detection of the carbapenemase GIM-1 in *Enterobacter cloacae* in Germany. J Antimicrob Chemother 68:558-61.

70. Huang S, Dai W, Sun S, Zhang X, Zhang L. 2012. Prevalence of plasmid-mediated quinolone resistance and aminoglycoside resistance determinants among carbapeneme non-susceptible *Enterobacter cloacae*. PLoS One 7, e47636. doi: 10.1371/journal.pone.0047636

71. Lee HK, Park YJ, Kim JY, Chang E, Cho SG, Chae HS, Kang CS. 2005. Prevalence of decreased susceptibility to carbapenems among *Serratia marcescens, Enterobacter cloacae,* and *Citrobacter freundii* and investigation of carbapenemases. Diagn Microbiol Infect Dis 52: 331-336.

72. Corkill JE, Anson JJ, Hart CA. 2005. High prevalence of the plasmid-mediated quinolone resistance determinant *qnrA* in multidrug-resistant *Enterobacteriaceae* from the blood cultures in Liverpool, UK. J Antimicrob Chemother 56:1115-1117.

73. Kanamori H, Yano H, Hirakata Y, Hirotani A, Arai K, Endo S, Ichimura S, Ogawa M, Shimojima M, Aoyagi T, Hatta M, Yamada M, Gu Y, Tokuda K, Kunishima H, Kitagawa M, Kaku M. 2012. Molecular characteristics of extended-spectrum β-lactamases and *qnr* determinants in *Enterobacter* species from Japan. PLoS One 7, e37967. doi: 10.1371/journal.pone.0037967.

74. Poirel L, Mammeri H, Nordmann P. 2004. TEM-121, a novel complex mutant of TEM-type beta-lactamase from *Enterobacter aerogenes*. Antimicrob Agents Chemother 48:4528-4531.

75. O'Hara CM, Steigerwalt AG, Hill BC, Farmer JJ III, Fanning GR, Brenner DJ. 1989. *Enterobacter hormaechei,* a new species of the family *Enterobacteriaceae* formerly known as enteric group 75. J Clin Microbiol 27:2046-2049.

76. Hoffmann H, Stindl S, Ludwig W, Stumpf A, Mehlen A, Monget D, Pierard D, Ziesing S, Heesemann J, Roggenkamp A, Schleifer KH. 2005. *Enterobacter hormaechei* subsp. *oharae* subsp. nov., *E. hormaechei* subsp. *hormaeche*i comb. nov., and *E. hormaechei* subsp. *steigerwaltii* subsp. nov., Three New Subspecies of clinical importance. J Clin Microbiol 43:3297-3303.

77. Hoffmann H, Schmoldt S, Trulzsch K, Stumpf A, Bengsch S, Blankenstein T, Heesemann J, Roggenkamp A. 2005. Nosocomial urosepsis caused by *Enterobacter kobei* with aberrant phenotype. Diagn Microbiol Infect Dis 53:143-147.

78. Wendel AF, Meyer S, Deenen R, Köhrer K, Kolbe-Busch S, Pfeffer K, Willmann M, Kaasch AJ, MacKenzie CR. 2018. Long-Term, Low-frequency cluster of a German-imipenemase-1-producing *Enterobacter hormaechei* ssp. *steigerwaltii* ST89 in a tertiary care hospital in Germany. Microb Drug Resist. doi: 10.1089/mdr.2017.0433. [Epub ahead of print]

79. Kosako YK, Tamura K, Sakazaki R, Miki K. 1996. *Enterobacter kobei* sp. nov., a new species of the family *Enterobacteriaceae* resembling *Enterobacter cloacae*. Curr Microbiol 33:261-265.

80. Zhou K, Yu W, Bonnet R, Cattoir V, Shen P, Wang B, Rossen JW, Xiao Y. 2017. Emergence of a novel *Enterobacter kobei* clone carrying chromosomal-encoded CTX-M-12 with diversified pathogenicity in northeast China. New Microbes New Infect 17:7-10.

81. Hoffmann H, Stindl S, Stumpf A, Mehlen A, Monget D, Heesemann J, Schleifer KH, Roggenkamp A. 2005. Description of *Enterobacter ludwigii* sp. nov., a novel *Enterobacter* species of clinical relevance. Syst Appl Microbiol 28:206-212.

82. Li G, Hu Z, Zeng P, Zhu B, Wu L. 2015. Whole genome sequence of *Enterobacter ludwigii* type strain EN-119T, isolated from clinical specimens. FEMS Microbiol Lett. 362(7). pii: fnv033. doi: 10.1093/femsle/fnv033. Epub 2015 Feb 26.

83. Flores-Carrero A, Labrador I, Paniz-Mondolfi A, Peaper DR, Towle D, Araque M. 2016. Nosocomial outbreak of extended-spectrum β-lactamase-producing *Enterobacter ludwigii* co-harbouring CTX-M-8, SHV-12 and TEM-15 in a neonatal intensive care unit in Venezuela. J Glob Antimicrob Resist 7:114-118. doi: 10.1016/j.jgar.2016.08.006.

84. Khajuria A, Praharaj AK, Grover N, Kumar M. 2013. First report of an *Enterobacter ludwigii* isolate coharboring NDM-1 and OXA-48 carbapenemases. Antimicrob Agents Chemother 57:5189-90. doi: 10.1128/AAC.00789-13.

85. Steigerwalt AG, Fanning GR, Fife-Asbury MA, Brenner DJ. 1976. DNA relatedness among species of *Enterobacter* and *Serratia*. Can J Microbiol 22:121-137.

86. Kim DM, Jang SJ, Neupane GP, Jang MS, Kwon SH, Kim SW, Kim WY. 2010. *Enterobacter nimipressuralis* as a cause of pseudobacteremia. BMC Infect Dis 10:315. doi: 10.1186/1471-2334-10-315.

87. Zhu B, Lou MM, Xie GL, Wang GF, Zhou Q, Wang F, Fang Y, Su T, Li B, Duan YP. 2011. *Enterobacter mori* sp. nov., associated with bacterial wilt on Morus alba L. Int J Syst Evol Microbiol 61(Pt 11):2769-74. doi: 10.1099/ijs.0.028613-0. Epub 2011 Jan 7.

88. Hartl R, Kerschner H, Gattringer R, Lepuschitz S, Allerberger F, Sorschag S, Ruppitsch W, Apfalter P. 2019. Whole-Genome Analysis of a Human *Enterobacter mori* Isolate Carrying a bla_{IMI-2} Carbapenemase in Austria. Microb Drug Resist 25:94-96. doi: 10.1089/mdr.2018.0098.

89. Izard D, Gavini F, Trinel PA, Krubwa F, Leclerc H. 1980. Contribution to DNA-DNA hybridization to the transfer of *Enterobacter aerogenes* to the genus *Klebsiella* as *K. mobilis*. Zentralbl Bakteriol Hyg 1: 257-263.

90. De Gheldre Y, Maes N, Rost F, De Ryck R, Clevenbergh P, Vincent JL, Struelens MJ. 1997. Molecular epidemiology of an outbreak of multidrug-resistant *Enterobacter aerogenes* infections and *in vivo* emergence of imipenem resistance. J Clin Microbiol 35:152-160.

91. Georghiou PR, Hamill RJ, Wright CE, Versalovic J, Koeuth T, Lupski J.R. 1995. Molecular epidemiology of infections due to *Enterobacter aerogenes*: identification of hospital outbreak-associated strains by molecular techniques. Clin Infect Dis 20:84-94.

92. Grattard F, Pozzetto B., Tabard L, Petit M, Ros A, Gaudin OG. 1995. Characterization of nosocomial strains of *Enterobacter aerogenes* by arbitrarily primed PCR analysis and ribotyping. Infect Control Hosp Epidemiol 16:224-230.

93. Jalaluddin S, Devaster JM, Scheen R, Gerard M, Butzler JP. 1998. Molecular epidemiological study of nosocomial *Enterobacter aerogenes* isolates in a Belgian hospital. J Clin Microbiol 36:1846-1852.

94. Lavigne JP, Sotto A, Nicolas-Chanoine MH, Bouziges N, Bourg G, Davin-Regli A, Pagès JM. 2012. Membrane permeability, a pivotal function involved in antibiotic resistance and virulence in *Enterobacter aerogenes* clinical isolates. Clin Microbiol Infect 18:539-545. doi: 10.1111/j.1469-0691.2011.03607.x.

95. Song E-H, K-H Park, E-Y Jang, E-J Lee, Y-P Chong, O-H Cho, S_H Kim, S-O Lee, H Sung, Kim MN, Jeong JY, Kim YS, Woo JH, Choi SH. 2010. Comparison of the clinical and microbiologic characteristics of patients with *Enterobacter cloacae* and *Enterobacter aerogenes* bacteremia: a prospective observation study. Diagnos Microbiol Infect Dis 66: 436-440.

96. Miro E, Alonso C, Navarro F, Mirelis B, Prats G. 1995. Resistencia al imipenem en *Enterobacter aerogenes*. Enferm Infecc Microbiol Clin 13:278-282.

97. Preston KE, Radomski CCA, Venezia RA. 2000. Nucleotide sequence of the chromosomal *ampC* gene of *Enterobacter aerogenes*. Antimicrob Agents Chemother 44:3158-3162.

98. Lee SH, Jeong SH, Park YM. 2003. Characterization of blaCMY-10 a novel, plasmid-encoded AmpC -type β-lactamase

gene in a clinical isolate of *Enterobacter aerogenes*. J Appl Microbiol 95:744-752.

99. Pitout JDD, Thomson KS, Hanson ND, Ehrhardt AF, Coudron P, Sanders CC. 1998. Plasmid-mediated resistance to expanded-spectrum cephalosporins among *Enterobacter aerogenes* strains. Antimicrob Agents Chemother 42:596-600.

100. Biendo M, Canarelli B, Thomas D, Rousseau F, Hamdad F, Adjide C, Laurans G, Eb F. 2008. Successive emergence of extended-spectrum beta-lactamase-producing and carbapenemase-producing *Enterobacter aerogenes* isolates in a university hospital. J Clin Microbiol 46:1037-1044. doi: 10.1128/JCM.00197-07.

101. Dumarche P, De Champs C, Sirot D, Chanal C, Bonnet R, Sirot J. 2002. TEM derivative-producing *Enterobacter aerogene*s strains: dissemination of a prevalent clone. Antimicrob. Agents Chemother 46:1128-1131.

102. Bousquet A, van der Mee-Marquet N, Dubost C, Bigaillon C, Larréché S, Bugier S, Surcouf C, Mérat S, Blanchard H, Mérens A. 2017. Outbreak of CTX-M-15-producing *Enterobacter cloacae* associated with therapeutic beds and syphons in an intensive care unit. Am J Infect Control 45:1160-1164.

103. Lavigne JP, Bouziges N, Chanal C, Mahamat A, Michaux-Charachon S, Sotto A. 2004. Molecular epidemiology of *Enterobacteriaceae* isolates producing extended-spectrum beta-lactamases in a French hospital. J Clin Microbiol 42:3805-3808.

104. Neuwirth C, Siebor E, Lopez J, Pechinot A, Kazmierczak A. 1996. Outbreak of TEM-24-producing *Enterobacter aerogenes* in an intensive care unit and dissemination of the extended-spectrum β-lactamase to other members of the family *Enterobacteriacae.* J Clin Microbiol 34:76-79.

105. Charrel RN, Pagès JM, De Micco P, Mallea M. 1996. Prevalence of outer membrane porin alteration in beta-lactam-antibiotic-resistant *Enterobacter aerogenes*. Antimicrob Agents Chemother 40:2854-2858.

106. Fernandez-Cuenca F, Rodriguez-Martinez JM, Martinez-Martinez L, Pascual A. 2006. In vivo selection of *Enterobacter aerogenes* with reduced susceptibility to cefepime and carbapenems associated with decreased expression of a 40 kDa outer membrane protein and hyperproduction of AmpC β–lactamase. Int J Antimicrob Agents 27:549-52.

107. Thiolas A, Bornet C, Davin-Régli A, Pagès JM, Bollet C. 2004. Resistance to imipenem, cefepime, and cefpirome associated with mutation in Omp36 osmoporin of *Enterobacter aerogenes*. Biochem Biophys Res Commun 317:851-856.

108. Dé E, Baslé A, Jaquinod M, Saint N, Malléa M, Molle G, Pagès JM. 2001. A new mechanism of antibiotic resistance in *Enterobacteriaceae* induced by a structural modification of the major porin. Mol Microbiol 41:189-198.

109. Chevalier J, Mulfinger C, Garnotel E, Nicolas P, Davin-Régli A, Pagès JM. 2008. Identification and evolution of drug efflux pump in clinical *Enterobacter aerogenes* strains isolated in 1995 and 2003. PLoS One 3:e3203. doi: 10.1371/journal.pone.0003203.

110. Thiolas A, Bollet C, La Scola B, Raoult D, Pagès JM. 2005 Successive emergence of *Enterobacter aerogenes* strains resistant to imipenem and colistin in a patient. Antimicrob Agents Chemother 49:1354-1358.

111. Richard C., Joly B, Sirot J, Stoleru GH, Popoff M. 1976. Étude de souches d'*Enterobacter* appartenant à un groupe particulier proche de *E. aerogenes*. Ann. Microbiol. Inst. Pasteur 127A:545-548.

112. Brenner DJ, C. Richard AG. Steigerwalt M, Asbury A, Mandel M. 1980. *Enterobacter gergoviae* sp. nov. : a new species of *Enterobacteriaceae* found in clinical specimens and the environment. Int J Syst Bacteriol 30:1-6.

113. Cheng Y, Chen M. 1994. Extended-spectrum beta-lactamases in clinical isolates of *Enterobacter gergoviae* and *Escherichia coli* in China. Antimicrob Agents Chemother 38:2838-42.

114. Freire MP, de Oliveira Garcia D, Cury AP, Spadão F, Di Gioia TS, Francisco GR, Bueno MF, Tomaz M, de Paula FJ, de Faro LB, Piovesan AC, Rossi F, Levin AS, David Neto E, Nahas WC, Pierrotti LC. 2016. Outbreak of IMP-producing carbapenem-resistant *Enterobacter gergoviae* among kidney transplant recipients. J Antimicrob Chemother 71:2577-85. doi: 10.1093/jac/dkw165.

115. Périamé M, Pagès JM, Davin-Regli A. 2015. *Enterobacter gergoviae* membrane modifications are involved in the adaptive response to preservatives used in cosmetic industry. J Appl Microbiol 118:49-61. doi: 10.1111/jam.12676.

116. Périamé M, Philippe N, Condell O, Fanning S, Pagès JM, Davin-Regli A. 2015. Phenotypic changes contributing to *Enterobacter gergoviae* biocide resistance. Lett Appl Microbiol 61:121-9. doi: 10.1111/lam.12435.

117. Davin-Regli A, Chollet R, Bredin J, Chevalier J, Lepine F, Pagès JM. 2006. *Enterobacter gergoviae* and the prevalence of efflux in parabens resistance. J Antimicrob Chemother 57:757-760.

118. Périamé M, Pagès JM, Davin-Regli A. 2014. *Enterobacter gergoviae* adaptation to preservatives commonly used in cosmetic industry. Int J Cosmet Sci 36:386-95. doi: 10.1111/ics.12140.

119. Doijad S, Imirzalioglu C, Yao Y, Pati NB, Falgenhauer L, Hain T, Foesel BU, Abt B, Overmann J, Mirambo MM, Mshana SE, Chakraborty T. 2016. *Enterobacter bugandensis* sp. nov., isolated from neonatal blood. Int J Syst Evol Microbiol 66:968-974. doi: 10.1099/ijsem.0.000821. Epub 2015 Dec 4.

120. Wu W, Feng Y, Zong Z. 2018. Characterization of a strain representing a new *Enterobacter* species, *Enterobacter chengduensis* sp. nov.2018. Antonie Van Leeuwenhoek. Oct 9. doi: 10.1007/s10482-018-1180-z. [Epub ahead of print].

121. Humann JL, Wildung M, Cheng CH, Lee T, Stewart JE, Drew JC, Triplett EW, Main D, Schroeder BK. 2011. Complete genome of the onion pathogen *Enterobacter cloacae* EcWSU1. Stand Genomic Sci. Dec 5:279-86. doi: 10.4056/sigs.2174950.

122. Richard C. 1989. Nouvelles *Enterobacteriaceae* rencontrées en bactériologie médicale : *Moellerella wisconsensis, Koserella trabulsii, Leclercia adercarboxylata, Escherichia fergusonii, Enterobacter asburiae, Rahnella aquatilis.* Ann Biol Clin 47:231-236.

123. Cordoba MA, Roccia IL, De Luca MM, Pezzani BC, Basualdo JA. 2001. Resistance to antibiotics in injured coliforms isolated from drinking water. Microbiol Immunol 45:383-386.

124. Dugleux G, Le Coutour X, Hecquard C, Oblin I. 1991. Septicemia caused by contaminated parenteral nutrition pouches: the refrigerator as an unusual cause. J Parent Ent Nutr 15:474-475.

125. Wang S.ATokars JI, Bianchine PJ, Carson LA, Arduino MJ, Smith AL, Hansen NC, Fitzgerald EA, Epstein JS, Jarvis WR.2000. *Enterobacter cloacae* bloodstream infections traced to contamined human albumine. Clinical Infectious Diseases 30:35-40.

126. Wang CC, Chu ML, Ho LJ, Hwang RC. 1991. Analysis of plasmid pattern in paediatric intensive care unit outbreaks of nosocomial infection due to *Enterobacter cloacae*.J Hosp Infect 19:33-40.

127. Mayhall CG, Lamb VA, Gayle WE Jr, Haynes BW Jr. 1979. *Enterobacter cloacae* septicemia in a burn center: epidemiology and control of an outbreak. J Infect Dis 139:166-71.

128. Farmer JJ III, Davis BR, Hickman-Brenner FW, McWorther A, Huntley-Carter GP, Asbury MA, Riddle C, Wathen-Grady HG, Elias C, Fanning GR, Steigerwalt AG, O'Hara CM, Morris GK, Smith PB, Brenner DJ. 1985. Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. J Clin Microbiol 21:46-76.

129. Garazzino S, Aprato A., Maiello A, Massé A, Biasibetti A, De Rosa FG, Di Perri G. 2005. Osteomyelitis caused by *Enterobacter cancerogenus* infection following a traumatic injury: case report and review of the literature. J Clin Microbiol 43:1459-1461.

130. Rubistein EM, Klevjer-Anderson P, Smith CA, Drouin MT, Evans Patterson J. 1993. *Enterobacter taylorae*, a new opportunistic pathogen: report of four cases. J Clin Microbiol 31:249-254.

131. Abbott S, Janda M. 1997. *Enterobacter cancerogenus ("Enterobacter taylorae*") infections associated with severe trauma or crush injuries. Mic Inf Dis 107:359-361.

132. Fernandez-Baca V, Ballesteros F, Hervas JA, Villalon P and Alberti S. 2001. Molecular epidemiological typing of *Enterobacter cloacae* isolates from a neonatal intensive care unit : three-year prospective study. J Hosp Infec 49: 173-182.

133. Fluit AC, Schmitz FJ, Verhoef J. 2001. Multi-resistance to antimicrobial agents for the ten most frequently isolated bacterial pathogens. Int J Antimicrob Agents 18:147-160.

134. Talon D, Menget P, Thouverez M, Thiriez G, Gbaguidi Haore H, Fromentin C, Muller A, Bertrand X. 2004. Emergence of *Enterobacter cloacae* as a common pathogen in neonatal units: pulse-field gel electrophoresis analysis. J Hosp Infect 57:119-125.

135. Fata F, Chittivelu S, Tessler S, Kupper Y. 1996. Gas gangrene of the arm due to *Enterobacter cloacae* in a neutropenic patient. South Med J 89:1095-1096.

136. Okhravi N, Ficker L, Matheson MM, Lightman S. 1998. *Enterobacter cloacae* endophtalmitis: report of four cases. J Clin Microbiol 36:48-51.

137. Saini AG, Rathore V, Ahuja CK, Chhabra R, Vaidya PC, Singhi P. 2017. Multiple brain abscesses due to *Enterobacter cloacae* in an immune-competent child. J Infect Public Health 10:674-677.

138. Chauhan S, Noor J, Yegneswaran B, Kodali H. 2016. *Enterobacter* Meningitis and Challenges in Treatment. J Clin Diagn Res 10(12):OD10-OD11. doi: 10.7860/JCDR/2016/20759.9081.

139. Jayaweera JA, Kothalawala M, Devakanthan B, Arunan S, Galgamuwa D, Rathnayake M. 2016. Spondylodiscitis Caused by *Enterobacter agglomerans*. Case Rep Infect Dis 2016:8491571.

140. Karasahin O, Yildiz Z, Unal O, Arslan U. 2017. A rare cause of healthcare-associated infective endocarditis: *Enterobacter cloacae*. Front Microbiol. 8:2343. doi: 10.3389/fmicb.2017.02343.

141. Arpin C, Dubois V, Coulange L, André C, Fischer I, Noury P, Grobost F, Brochet JP, Jullin J, Dutilh B, Larribet G, Lagrange I, Quentin C. 2003. Extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in community and private health care centers. Antimicrob Agents Chemother 47:3506-14.

142. Bouraoui H, Trimeche B, Kaabia N, Zaaraoui J, Mahdhaoui A, Ernez-Hajri S, Jeridi G, Ammar H. 2004. Endocardite infectieuse à *Enterobacter aerogenes*. La revue de médecine interne 26:71-80.

143. Ganeswire R, Thong K L, Pthucheary SD. 2003. Nosocomial outbreak of *Enterobacter gergoviae* bacteraemia in a neonatal intensive care unit. J Hosp Infect 53:292-296.

144. Kesieme EB, Kesieme CN, Akpede GO, Okonta KE, Dongo AE, Gbolagade AM, Eluenhike SU. 2012. Tension pneumatocele due to *Enterobacter gergoviae* pneumonia: a case report. Case Report Med :808630. doi: 10.1155/2012/808630.

145. Haiko J, Westerlund-Wikström B. 2013. The role of the bacterial flagellum in adhesion and virulence. Biology (Basel) 2:1242-67. doi: 10.3390/biology2041242.

146. Barnes AI, Ortiz C, Paraje MG, Balanzino LE, Albesa I. 1997. Purification and characterization of a cytotoxin from *Enterobacter cloacae*. Can J Microbiol 43:729–733.

147. Krzymińska S, Mokracka J, Koczura R, Kaznowski A. 2009. Cytotoxic activity of *Enterobacter cloacae* human isolates. FEMS Immunol Med Microbiol 56:248-52. doi: 10.1111/j.1574-695X.2009.00572.x.

148. Krzymińska S, Koczura R, Mokracka J, Puton T, Kaznowski A. 2010. Isolates of the *Enterobacter cloacae* complex induce apoptosis of human intestinal epithelial cells. Microb. Pathog 49:83-89.

149. Souza Lopes AC, Rodrigues JF, Cabral AB, da Silva ME, Leal NC, da Silveira VM, de Morais Júnior MA. 2016. Occurrence and analysis of *irp2* virulence gene in isolates of *Klebsiella pneumoniae* and *Enterobacter* spp. from microbiota and hospital and community-acquired infections. Microb Pathog 96:15–19.

150. Kim S-M, Lee H-W, Choi Y-W, Kim S-H, Lee J-C, Lee Y-C, Seol S-Y, Cho D-T, Kim J. 2012. Involvement of curli fimbriae in the biofilm formation of *Enterobacter cloacae*. J. Microbiol. Seoul Korea 50:175-178.

151. Azevedo PAA, Furlan JPR, Oliveira-Silva M, Nakamura-Silva R, Gomes CN, Costa KRC, Stehling EG, Pitondo-Silva A. 2018. Detection of virulence and β-lactamase encoding genes in *Enterobacter aerogenes* and *Enterobacter cloacae* clinical isolates from Brazil. Braz J Microbiol 49 Suppl 1:224-228. doi: 10.1016/j.bjm.2018.04.009.

152. El Fertas-Aissani R, Messai Y, Alouache S, Bakour R. 2013. Virulence profiles and antibiotic susceptibility patterns of *Klebsiella pneumoniae* strains isolated from different clinical specimens. Pathol Biol (Paris) 61:209-16. doi: 10.1016/j.patbio.2012.10.004.

153. Compain F, Babosan A, Brisse S, Genel N, Audo J, Ailloud F, Kassis-Chikhani N, Arlet G, Decré D. 2014. Multiplex PCR for detection of seven virulence factors and K1/K2 capsular serotypes of *Klebsiella pneumoniae*. J Clin Microbiol 52:4377-80. doi: 10.1128/JCM.02316-14. Epub 2014 Oct 1.

154. Lavigne JP, Sotto A, Nicolas-Chanoine MH, Bouziges N, Pagès JM, Davin-Regli A. 2013. An adaptive response of *Enterobacter aerogenes* to imipenem: regulation of porin balance in clinical isolates. Int J Antimicrob Agents 41:130-136. doi: 10.1016/j.ijantimicag.2012.10.010.

155. Rostad CA, Wehrheim K, Kirklin JK, Naftel D, Pruitt E, Hoffman TM, L'Ecuyer T, Berkowitz K, Mahle WT, Scheel JN. 2017. Bacterial infections after pediatric heart transplantation: Epidemiology, risk factors and outcomes. J Heart Lung Transplant. 36:996-1003. doi: 10.1016/j.healun.2017.05.009.

156. Huh K, Kang CI, Kim J, Cho SY, Ha YE, Joo EJ, Chung DR, Lee NY, Peck KR, Song JH. 2014. Risk factors and treatment outcomes of bloodstream infection caused by extended-spectrum cephalosporin-resistant Enterobacter species in adults with cancer. Diagn Microbiol Infect Dis 78:172-7. doi: 10.1016/j.diagmicrobio.2013.11.002.

157. Gaynes RP, Edwards JR, Jarvis WR, Culver DH, Tolson JS, Martone WJ. 1996. Nosocomial infections among neonates in high-risk nurseries in the United States. National Nosocomial Infections Surveillance System. Pediatrics. 98(3 Pt 1):357-361.

158. Vidal-Navarro L, Pfeiffer C, Bouziges N, Sotto A, Lavigne JP. 2010. Faecal carriage of multidrug-resistant Gram-negative bacilli during a non-outbreak situation in a French university hospital. J Antimicrob Chemother. 65:2455-8. doi: 10.1093/jac/dkq333.

159. Boutet-Dubois A, Pantel A, Prère MF, Bellon O, Brieu-Roche N, Lecaillon E, Le Coustumier A, Davin-Regli A, Villeneuve L, Bouziges N, Gleize E, Lamarca R, Dunyach-Remy C, Sotto A, Lavigne JP. 2013. Faecal carriage of oxyiminocephalosporin-resistant *Enterobacteriaceae* among paediatric units in different hospitals in the south of France. Eur J Clin Microbiol Infect Dis 32:1063-8. doi: 10.1007/s10096-013-1851-7. Epub 2013 Mar 15.

160. Arpin C, Dubois V, Maugein J, Jullin J, Dutilh B, Brochet JP, Larribet G, Fischer I, Quentin C. 2005. Clinical and molecular analysis of extended-spectrum β-lactamase-producing enterobacteria in the community setting. J Clin Microbiol 43:5048-5054.

161. Lee EH, Collatz E, Trias J, Gutmann L. 1992 Diffusion of beta-lactam antibiotics into proteoliposomes reconstituted with outer membranes of isogenic imipenem-susceptible and -resistant strains of *Enterobacter cloacae*. J Gen Microbiol 138:2347-2351.

162. Tebano G, Geneve C, Tanaka S, Grall N, Atchade E, Augustin P, Thabut G, Castier Y, Montravers P, Desmard M. 2016. Epidemiology and risk factors of multidrug-resistant bacteria in respiratory samples after lung transplantation. Transpl Infect Dis 18:22-30. doi: 10.1111/tid.12471.

163. Hyle EP, Ferraro MJ, Silver M, Lee H, Hooper DC. 2010. Ertapenem-resistant *Enterobacteriaceae*: risk factors for acquisition and outcomes. Infect Control Hosp Epidemiol 31:1242-9. doi: 10.1086/657138.

163. Ivády B, Kenesei É, Tóth-Heyn P, Kertész G, Tárkányi K, Kassa C, Ujhelyi E, Mikos B, Sápi E, Varga-Heier K, Guóth G, Szabó D. 2016. Factors influencing antimicrobial resistance and outcome of Gram-negative bloodstream infections in children. Infection 44:309-21. doi: 10.1007/s15010-015-0857-8.

165. Mezzatesta ML, Gona F, Stefani S. 2012. *Enterobacter cloacae* complex: clinical impact and emerging antibiotic resistance. Future Microbiol 7:887-902. doi: 10.2217/fmb.12.61.

166. Dalben M, Varkulja G, Basso M, Krebs VLJ, Gibelli MA, van der Heijden I, Rossi F, Duboc G, Levin AS, Costa SF. 2008. Investigation of an outbreak of *Enterobacter cloacae* in a neonatal unit and review of the literature. J Hosp Infect 70:7–14.

167. Campos LC, Lobianco LF, Seki LM, Santos RMR, Asensi MD: Outbreak of *Enterobacter hormaechei* septicaemia in newborns caused by contaminated parenteral nutrition in Brazil. 2007. J Hosp Infect 66:95–97.

168. Tuon FF, Cieslinski J, Ono AFM, Goto FL, Machinski JM, Mantovani LK, Kosop LR, Namba MS, Rocha JL. 2018. Microbiological profile and susceptibility pattern of surgical site infections related to orthopaedic trauma. Int Orthop. Aug 2. doi: 10.1007/s00264-018-4076-7. [Epub ahead of print]

169. Kalakouti E, Simillis C, Pellino G, Mughal N, Warren O, Mills S, Tan E, Kontovounisios C, Tekkis PP. 2017. Characteristics of surgical site infection following colorectal surgery in a tertiary center: extended-spectrum β-lactamase-producing bacteria culprits in disease. Wounds 30:108-113.

170. Alexiou K, Drikos I, Terzopoulou M, Sikalias N, Ioannidis A, Economou N. 2017. A prospective randomised trial of isolated pathogens of surgical site infections (SSI). Ann Med Surg (Lond). 21:25-29. doi: 10.1016/j.amsu.2017.07.045. eCollection 2017 Sep.

171. Scheufele F, Aichinger L, Jäger C, Demir IE, Schorn S, Sargut M, Erkan M, Kleeff J, Friess H, Ceyhan GO. 2017. Effect of preoperative biliary drainage on bacterial flora in bile of patients with periampullary cancer. Br J Surg 104:e182-e188. doi: 10.1002/bjs.10450.

172. Chen H, Zhang Y, Chen YG, Yu YS, Zheng SS, Li LJ. Sepsis resulting from *Enterobacter aerogenes* resistant to carbapenems after liver transplantation. Hepatobiliary Pancreat Dis Int. 2009 Jun;8(3):320-322.

173. Hadano Y, Tamagawa K, Ohkusu K. 2018. Trauma wound related infection caused by *Enterobacter cancerogenus* and *Aeromonas hydrophilia*. Intern Med. 57:131-133. doi: 10.2169/internalmedicine.9171-17. Epub 2017 Oct 16.

174. Kremer A, Hoffmann H. 2012. Prevalences of the *Enterobacter cloacae* complex and its phylogenetic derivatives in the nosocomial environment. Eur J Clin Microbiol Infect Dis 31:2951–2955.

175. Jneid J, Cassir N, Schuldiner S, Jourdan N, Sotto A, Lavigne JP, La Scola B. 2018. Exploring the microbiota of diabetic foot infections with culturomics. Front Cell Infect Microbiol. 8:282. doi: 10.3389/fcimb.2018.00282. eCollection.

176. Jacobson KL, Cohen SH, Inciardi JF, King JH, Lippert WE, Iglesias T, VanCouwenberghe CJ. 1995. The relationship between antecedent antibiotic use and resistance to extended-spectrum cephalosporins in group I b-lactamase-producing organisms. Clin Infect Dis 21:1107-1113.

177. Segreti J, Levin S. 1996. Bacteriologic and clinical applications of a new extended-spectrum parental cephalosporin. Am J Med 100:45S.1991;35:976-82.

178. Fung-Tomc J, Dougherty TJ, DeOrio FJ, Simich-Jacobson V, Kessler RE. 1989. Activity of cefepime against ceftazidime- and cefotaxime-resistant gram-negative bacteria and its relationship to beta-lactamase levels. Antimicrob Agents Chemother 33:498-502.

179. McKamey L, Venugopalan V, Cherabuddi K, Borgert S, Voils S, Shah K, Klinker KP. 2018. Assessing antimicrobial stewardship initiatives: Clinical evaluation of cefepime or piperacillin/tazobactam in patients with bloodstream infections secondary to AmpC-producing organisms. Int J Antimicrob Agents. 52:719-723. doi: 10.1016/j.ijantimicag.2018.08.007. Epub 2018 Aug 17.

180. Cheng L, Nelson BC, Mehta M, Seval N, Park S, Giddins MJ, Shi Q, Whittier S, Gomez-Simmonds A, Uhlemann AC. 2017. Piperacillin-tazobactam versus other antibacterial agents for treatment of bloodstream infections due to AmpC β-lactamase-producing *Enterobacteriaceae*. Antimicrob Agents Chemother 61(6). pii: e00276-17. doi: 10.1128/AAC.00276-17.

181. Sanders WE, Tenney JH, Kessler RE. 1996. Efficacy of cefepime in the treatment of infections due to multiply resistant *Enterobacter* species. Clin Infect Dis 23:454-61.

182. Harris PN, Wei JY, Shen AW, Abdile AA, Paynter S, Huxley RR, Pandeya N, Doi Y, Huh K, O'Neal CS, Talbot TR, Paterson DL. 2016. Carbapenems versus alternative antibiotics for the treatment of bloodstream infections caused by *Enterobacter*, *Citrobacter* or *Serratia* species: a systematic review with meta-analysis. J Antimicrob Chemother 71:296-306. doi: 10.1093/jac/dkv346.

183. Dobias J, Dénervaud-Tendon V, Poirel L, Nordmann P. 2017. Activity of the novel siderophore cephalosporin cefiderocol against multidrug-resistant Gram-negative pathogens. Eur J Clin Microbiol Infect Dis 36:2319-2327. doi: 10.1007/s10096-017-3063-z. Epub 2017 Jul 26.

184. Livermore DM, Mushtaq S, Warner M, Vickers A, Woodford N. 2017. In vitro activity of cefepime/zidebactam (WCK 5222) against Gram-negative bacteria. J Antimicrob Chemother. 72:1373-1385. doi: 10.1093/jac/dkw593.

185. Sader HS, Castanheira M, Mendes RE, Flamm RK, Jones RN. 2017. Antimicrobial activity of high-proportion cefepime-tazobactam (WCK 4282) against a large number of Gram-negative isolates collected worldwide in 2014. Antimicrob Agents Chemother 61(4). pii: e02409-16. doi: 10.1128/AAC.02409-16.

186. Mischnik A, Baumert P, Hamprecht A, Rohde A, Peter S, Feihl S, Knobloch J, Gölz H, Kola A, Obermann B, Querbach C, Willmann M, Gebhardt F, Tacconelli E, Gastmeier P, Seifert H, Kern WV; DZIF-ATHOS Study Group. 2017.

Susceptibility to cephalosporin combinations and aztreonam/avibactam among third-generation cephalosporin-resistant *Enterobacteriaceae* recovered on hospital admission. Int J Antimicrob Agents. 49:239-242. doi: 10.1016/j.ijantimicag.2016.10.013.

187. Haidar G, Alkroud A, Cheng S, Churilla TM, Churilla BM, Shields RK, Doi Y, Clancy CJ, Nguyen MH. 2016. Association between the presence of aminoglycoside-modifying enzymes and in vitro activity of Gentamicin, Tobramycin, Amikacin, and Plazomicin against *Klebsiella pneumoniae* carbapenemase- and extended-spectrum-β-lactamase-producing Enterobacter species. Antimicrob Agents Chemother 60:5208-14. doi: 10.1128/AAC.00869-16.

188. Boucher HW, Talbot GH, Benjamin DK Jr, Bradley J, Guidos RJ, Jones RN, Murray BE, Bonomo RA, Gilbert D, Infectious Diseases Society of America. 2013. 10 x '20 Progress--development of new drugs active against Gram-negative bacilli: an update from the Infectious Diseases Society of America. Clin Infect Dis 56:1685-1694. doi: 10.1093/cid/cit152.

189. Nikaido H. 2003. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 67:593–656.

190. Pagès JM, James CE, Winterhalter M. 2008. The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. Nat Rev Microbiol 6:893–903.

191. Delcour AH. 2009. Outer membrane permeability and antibiotic resistance. Biochim Biophys Acta 1794:808-816. doi: 10.1016/j.bbapap.2008.11.005.

192. Zgurskaya HI, Löpez CA, Gnanakaran S. 2015. Permeability barrier of Gram-negative cell envelopes and approaches to bypass it. ACS Infect Dis 1:512–522.

193. Nikaido H. 1985. Role of permeability barriers in resistance to ß-lactams antibiotics. Pharmac Ther 27:197-231.

194. Nikaido H. 1989. Outer membrane barrier as a mechanism of antimicrobial resistance. Antimicrob Agents Chemother 33:1831–1836.

195. Valade E, Davin-Regli A, Bolla JM, Pagès JM. 2014. Bacterial Membrane, a key for controlling drug influx and efflux, pp217-240. In Gualerzi CO, Brandi L, Fabbretti A, Pon CL (ed), In Antibiotics - Targets, Mechanims and Resistance. Wiley-VCH Verlag GmbH & Co. KGaA Publications.

196. Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ. 2015. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 13:42-51. doi: 10.1038/nrmicro3380.

197. Bush K. 2018. Past and present perspectives on β-lactamases. Antimicrob Agents Chemother 62:pii:e01076-18. doi: 10.1128/AAC.01076-18.

198. Carter MQ, Pham A, Huynh S, He X. 2017. Complete Genome Sequence of a Shiga Toxin-producing *Enterobacter cloacae* clinical isolate. Genome Announc 5(37). pii: e00883-17. doi: 10.1128/genomeA.00883-17.

199. Martínez-Martínez L. 2008. Extended-spectrum beta-lactamases and the permeability barrier. Clin Microbiol Infect 14:82-89.

200. Partridge SR, Kwong SM, Firth N, Jensen SO. 2018. Mobile genetic elements associated with antimicrobial resistance. Clin Microbiol Rev 31. pii: e00088-17.doi: 10.1128/CMR.00088-17.

201. Malléa M, Chevalier J, Bornet C, Eyraud A, Davin-Regli A, Bollet C, Pagès JM. 1998. Porin alteration and active efflux: two in vivo drug resistance strategies used by *Enterobacter aerogenes*. Microbiology 144:3003-3009.

202. Babouee Flury B, Ellington MJ, Hopkins KL, Turton JF, Doumith M, Woodford N. 2016. The differential importance of mutations within AmpD in cephalosporin resistance of *Enterobacter aerogenes* and *Enterobacter cloacae*. Int J Antimicrob Agents 48:555-558. doi: 10.1016/j.ijantimicag.2016.07.021.

203. Jean SS, Hsueh PR, and SMART Asia-Pacific Group. 2017 Distribution of ESBLs, AmpC β-lactamases and carbapenemases among *Enterobacteriaceae* isolates causing intra-abdominal and urinary tract infections in the Asia-Pacific region during 2008-14: results from the Study for Monitoring Antimicrobial Resistance Trends (SMART). J Antimicrob Chemother 72:166-171.

204. Zhou K, Yu W, Cao X, Shen P, Lu H, Luo Q, Rossen JWA, Xiao Y. 2018. Characterization of the population structure, drug resistance mechanisms and plasmids of the community-associated *Enterobacter cloacae* complex in China. J Antimicrob Chemother. 73:66-76. doi: 10.1093/jac/dkx361.

205. Lee SO, Kim YS, Kim BN, Kim MN, Woo JH, Ryu J. 2002. Impact of previous use of antibiotics on development of resistance to extended-spectrum cephalosporins in patients with *Enterobacter* bacteremia. Eur J Clin Microbiol Infect Dis 21:577-81.

206. Jarlier V and INVS. 2014. Surveillance of multidrug resistant bacteria in French healthcare Facilities *BMR-Raisin network* Données 2012 Saint-Maurice: Institut de veille sanitaire. http://www.invs.sante.fr.

207. Siebor E, de Curraize C, Neuwirth C. 2018. Identification of AGI1-A, a variant of Acinetobacter genomic island 1 (AGI1), in a French clinical isolate belonging to the Enterobacter cloacae complex. J Antimicrob Chemother Nov 8. doi: 10.1093/jac/dky442. [Epub ahead of print]

208. Torres E, López-Cerero L, Del Toro MD, Pascual A. 2014. First detection and characterization of an OXA-48-producing *Enterobacter aerogenes* isolate. Enferm Infecc Microbiol Clin 32:469-70.

209. Khajuria A, Praharaj AK, Kumar M, Grover N. 2014. Carbapenem resistance among *Enterobacter* species in a tertiary care hospital in central India. Chemother Res Pract 2014:972646. doi: 10.1155/2014/972646.

210. Beyrouthy R, Barets M, Marion E, Dananché C, Dauwalder O, Robin F, Gauthier L, Jousset A, Dortet L, Guérin F, Bénet T, Cassier P, Vanhems P, Bonnet R. 2018. Novel *Enterobacter* lineage as leading cause of nosocomial outbreak involving carbapenemase-Producing Strains. Emerg Infect Dis 24:1505-1515. doi: 10.3201/eid2408.180151.

211. Boyd DA, Mataseje LF, Davidson R, Delport JA, Fuller J, Hoang L, Lefebvre B, Levett PN, Roscoe DL, Willey BM, Mulvey MR. 2017. *Enterobacter cloacae* complex isolates harboring $bla_{\text{NMC-A}}$ or bla_{IMI}-type class A carbapenemase genes on novel chromosomal integrative elements and plasmids. Antimicrob Agents Chemother 61(5). pii: e02578-16. doi: 10.1128/AAC.02578-16.

212. Kubota H, Uwamino Y, Matsui M, Sekizuka T, Suzuki Y, Okuno R, Uchitani Y, Ariyoshi T, Aoki W, Suzuki S, Kuroda M, Shinkai T, Yokoyama K, Sadamasu K, Funakoshi T, Murata M, Hasegawa N, Iwata S. 2018. FRI-4 carbapenemase-producing *Enterobacter cloacae* complex isolated in Tokyo, Japan. J Antimicrob Chemother. doi: 10.1093/jac/dky291. [Epub ahead of print]

213. Meunier D, Findlay J, Doumith M, Godoy D, Perry C, Pike R, Gronthoud F, Shryane T, Poirel L, Welfare W, Woodford N, Hopkins KL. 2017. FRI-2 carbapenemase-producing *Enterobacter cloacae* complex in the UK. J Antimicrob Chemother. 72:2478-2482.

214. Potron A, Poirel L, Rondinaud E, Nordmann P. 2013. Intercontinental spread of OXA-48 β-lactamase-producing *Enterobacteriaceae* over a 11-year period, 2001 to 2011. Euro Surveill. 18. pii: 20549.

215. Izdebski R, Baraniak A, Zabicka D, Sekowska A, Gospodarek-Komkowska E, Hryniewicz W, Gniadkowski M. 2018. VIM/IMP carbapenemase-producing *Enterobacteriaceae* in Poland: epidemic *Enterobacter hormaechei* and *Klebsiella oxytoca* lineages. J Antimicrob Chemother Jul 6. doi: 10.1093/jac/dky257. [Epub ahead of print]

216. Karlowsky JA, Lob SH, Kazmierczak KM, Badal RE, Young K, Motyl MR, Sahm DF. 2017. *In vitro* activity of imipenem against carbapenemase-positive *Enterobacteriaceae* isolates collected by the SMART Global Surveillance Program from 2008 to 2014. J Clin Microbiol 55:1638-1649. doi: 10.1128/JCM.02316-16.

217. Chavda KD, Chen L, Fouts DE, Sutton G, Brinkac L, Jenkins SG, Bonomo RA, Adams MD, Kreiswirth BN. 2016. Comprehensive genome analysis of carbapenemase-producing *Enterobacter* spp.: new insights into phylogeny, population structure, and resistance mechanisms. MBio 7. pii: e02093-16. doi: 10.1128/mBio.02093-16.

218. Wang Q, Wang X, Wang J, Ouyang P, Jin C, Wang R, Zhang Y, Jin L, Chen H, Wang Z, Zhang F, Cao B, Xie L, Liao K, Gu B, Yang C, Liu Z, Ma X, Jin L, Zhang X, Man S, Li W, Pei F, Xu X, Jin Y, Ji P, Wang H. 2018. Phenotypic and Genotypic Characterization of Carbapenem-resistant *Enterobacteriaceae*: Data From a Longitudinal Large-scale CRE Study in China (2012-2016). Clin Infect Dis 67(suppl_2):S196-S205. doi: 10.1093/cid/ciy660.

219. Wilson BM, El Chakhtoura NG, Patel S, Saade E, Donskey CJ, Bonomo RA, Perez F. 2017. Carbapenem-Resistant *Enterobacter cloacae* in Patients from the US Veterans Health Administration, 2006-2015. Emerg Infect Dis 23:878-880. doi: 10.3201/eid2305.162034.

220. Peirano G, Matsumura Y, Adams MD, Bradford P, Motyl M, Chen L, Kreiswirth BN, Pitout JDD. 2018. Genomic Epidemiology of Global Carbapenemase-Producing *Enterobacter* spp, 2008-2014. Emerg Infect Dis 24:1010-1019. doi: 10.3201/eid2406.171648.

221. Gomez-Simmonds A, Stump S, Giddins MJ, Annavajhala MK, Uhlemann AC. 2018. Clonal background, resistance gene profile, and porin gene mutations modulate *in vitro* susceptibility to imipenem-relebactam in diverse *Enterobacteriaceae*. Antimicrob Agents Chemother 62. pii: e00573-18. doi: 10.1128/AAC.00573-18.

222. Miró E, Grünbaum F, Gómez L, Rivera A, Mirelis B, Coll P, Navarro F. 2013. Characterization of aminoglycoside-modifying enzymes in *Enterobacteriaceae* clinical strains and characterization of the plasmids implicated in their diffusion. Microb Drug Resist 19:94-99. doi: 10.1089/mdr.2012.0125.

223. Khennouchi NC, Loucif L, Boutefnouchet N, Allag H, Rolain JM. 2015. MALDI-TOF MS as a tool to detect a nosocomial outbreak of extended-spectrum-β-lactamase- and ArmA methyltransferase-producing *Enterobacter cloacae* clinical isolates in Algeria. Antimicrob Agents Chemother 59:6477-6483. doi: 10.1128/AAC.00615-15.

224. Bakthavatchalam YD, Pragasam AK, Biswas I, Veeraraghavan B. 2018. Polymyxin susceptibility testing, interpretative breakpoints and resistance mechanisms: An update. J Glob Antimicrob Resist 12:124-136. doi: 10.1016/j.jgar.2017.09.011.

225. Ebbensgaard A, Mordhorst H, Aarestrup FM, Hansen EB. 2018. The Role of Outer Membrane Proteins and Lipopolysaccharides for the Sensitivity of *Escherichia coli* to Antimicrobial Peptides. Front Microbiol 9:2153. doi: 10.3389/fmicb.2018.02153.

226. Braun G, Cole ST. 1983. Molecular characterization of the gene coding for major outer membrane protein OmpA from *Enterobacter aerogenes*. Eur J Biochem 137:495-500.

227. Nieweg A, Bremer E. 1997. The nucleoside-specific Tsx channel from the outer membrane of *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Enterobacter aerogenes*: functional characterization and DNA sequence analysis of the *tsx* genes. Microbiology 143:603-615.

228. Schulz GE. 2002. The structure of bacterial outer membrane proteins. Biochim Biophys Acta 1565:308-317.

229. Fairman JW, Noinaj N, Buchanan SK. 2011. The structural biology of β-barrel membrane proteins: a summary of recent reports. Curr Opin Struct Biol 21:523-531. doi: 10.1016/j.sbi.2011.05.005.

230. Selkrig J, Leyton DL, Webb CT, Lithgow T. 2014. Assembly of β-barrel proteins into bacterial outer membranes. Biochim Biophys Acta 1843:1542-1550. doi: 10.1016/j.bbamcr.2013.10.009.

231. Sperandeo P, Martorana AM, Polissi A. 2017. Lipopolysaccharide biogenesis and transport at the outer membrane of Gram-negative bacteria. Biochim Biophys Acta Mol Cell Biol Lipids 1862:1451-1460. doi: 10.1016/j.bbalip.2016.10.006.

232. Acosta-Gutiérrez S, Ferrara L, Pathania M, Masi M, Wang J, Bodrenko I, Zahn M, Winterhalter M, Stavenger RA, Pagès JM, Naismith JH, van den Berg B, Page MGP, Ceccarelli M. 2018. Getting Drugs into Gram-Negative Bacteria: Rational Rules for Permeation through General Porins. ACS Infect Dis 4:1487-1498. doi: 10.1021/acsinfecdis.8b00108.

233. James CE, Mahendran KR, Molitor A, Bolla JM, Bessonov AN, Winterhalter M, Pagès JM. 2009. How beta-lactam antibiotics enter bacteria: a dialogue with the porins. PLoS One 4:e5453. doi: 10.1371/journal.pone.0005453.

234. Chevalier J, Malléa M, Pagès JM. 2000. Comparative aspects of the diffusion of norfloxacin, cefepime and spermine through the F porin channel of *Enterobacter cloacae*. Biochem J 348:223-227.

235. Malléa M, Simonet V, Lee EH, Gervier R, Collatz E, Gutmann L, Pagès JM. 1995. Biological and immunological comparisons of *Enterobacter cloacae* and *Escherichia coli porins*. FEMS Microbiol Lett 129:273-279.

236. De la Cruz MA, Calva E. 2010. The complexities of porin genetic regulation. J Mol Microbiol Biotechnol 18:24-36. doi: 10.1159/000274309.

237.Masi M, Winterhalter M, Pagès JM. 2019. Outer membrane porins. In Bacterial Cell Walls and Membranes, Ed A. Kuhn. Springer Nature publications, pp1-54.

238. Bornet C, Chollet R, Malléa M, Chevalier J, Davin-Regli A, Pagès JM, Bollet C. 2003. Imipenem and expression of multidrug efflux pump in *Enterobacter aerogenes.* Biochem Biophys Res Commun 301:985-990.

239. Philippe N, Maigre L, Santini S, Pinet E, Claverie JM, Davin-Régli AV, Pagès JM, Masi M. 2015. In vivo evolution of bacterial resistance in two cases of *Enterobacter aerogenes* infections during treatment with imipenem. PLoS One 10:e0138828. doi: 10.1371/journal.pone.0138828.

240. Gayet S, Chollet R, Molle G, Pagès JM, Chevalier J. 2003. Modification of outer membrane protein profile and evidence suggesting an active drug pump in *Enterobacter aerogenes* clinical strains. Antimicrob Agents Chemother 47:1555-1559.

241. Dupont M, James CE, Chevalier J, Pagès JM. 2007. An early response to environmental stress involves regulation of OmpX and OmpF, two enterobacterial outer membrane pore-forming proteins. Antimicrob Agents Chemother 51:3190-3198.

242. Stoorvogel J, van Bussel MJ, Tommassen J, van de Klundert JA. 1991. Molecular characterization of an *Enterobacter cloacae* outer membrane protein (OmpX). J Bacteriol 173:156-160.

243. Stoorvogel J, van Bussel MJ, van de Klundert JA. 1991. Biological characterization of an *Enterobacter cloacae* outer membrane protein (OmpX). J Bacteriol 173:161-167.

244. Bauer K, Schmid A, Boos W, Benz R, Tommassen J. 1988. Pore formation by pho-controlled outer-membrane proteins of various *Enterobacteriaceae* in lipid bilayers. Eur J Biochem 174:199-205.

245. Bornet C, Saint N, Fetnaci L, Dupont M, Davin-Régli A, Bollet C, Pagès JM. 2004. Omp35, a new *Enterobacter aerogenes* porin involved in selective susceptibility to cephalosporins. Antimicrob Agents Chemother 48:2153-2158.

246. Doumith M, Ellington MJ, Livermore DM, Woodford N. 2009. Molecular mechanisms disrupting porin expression in ertapenem-resistant *Klebsiella* and *Enterobacter* spp. clinical isolates from the UK. J Antimicrob Chemother 63:659-667. doi: 10.1093/jac/dkp029.

247. Dupont H, Gaillot O, Goetgheluck AS, Plassart C, Emond JP, Lecuru M, Gaillard N, Derdouri S, Lemaire B, Girard de Courtilles M, Cattoir V, Mammeri H. 2015. Molecular characterization of carbapenem-nonsusceptible enterobacterial isolates collected during a prospective interregional survey in france and susceptibility to the novel ceftazidime-avibactam and aztreonam-avibactam combinations. Antimicrob Agents Chemother 60:215-221. doi: 10.1128/AAC.01559-15.

248. Babouee Flury B, Ellington MJ, Hopkins KL, Turton JF, Doumith M, Loy R, Staves P, Hinic V, Frei R, Woodford N. 2016. Association of novel nonsynonymous single sucleotide polymorphisms in AmpD with cephalosporin resistance and phylogenetic variations in AmpC, AmpR, OmpF, and OmpC in *Enterobacter cloacae* isolates that are highly resistant to carbapenems. Antimicrob Agents Chemother 60:2383-2390. doi: 10.1128/AAC.02835-15.

249. Bedenić B, Vranić-Ladavac M, Venditti C, Tambić-Andrašević A, Barišić N, Gužvinec M, Karčić N, Petrosillo N, Ladavac R, di Caro A. 2018. Emergence of colistin resistance in *Enterobacter aerogenes* from Croatia. J Chemother 30:120-123. doi: 10.1080/1120009X.2017.1382121.

250. Hao M, Ye M, Shen Z, Hu F, Yang Y, Wu S, Xu X, Zhu S, Qin X, Wang M. 2018. Porin deficiency in carbapenem-resistant *Enterobacter aerogenes* strains. Microb Drug Resist 24:1277-1283. doi: 10.1089/mdr.2017.0379.

251. Phan K, Ferenci T. 2017. The fitness costs and trade-off shapes associated with the exclusion of nine antibiotics by OmpF porin channels. ISME J 11:1472-1482. doi: 10.1038/ismej.2016.202.

252. Hennequin C, Robin F. 2016. Correlation between antimicrobial resistance and virulence in *Klebsiella pneumoniae*. Eur J Clin Microbiol Infect Dis 35:333-341. doi: 10.1007/s10096-015-2559-7.

253. Ferreira RL, da Silva BCM, Rezende GS, Nakamura-Silva R, Pitondo-Silva A, Campanini EB, Brito MCA, da Silva EML, Freire CCM, da Cunha AF, Pranchevicius MDS. High Prevalence of Multidrug-Resistant *Klebsiella pneumoniae* Harboring Several Virulence and β-Lactamase Encoding Genes in a Brazilian Intensive Care Unit.Front Microbiol. 2019 Jan 22;9:3198. doi: 10.3389/fmicb.2018.03198. eCollection 2018.

254. Pantel A, Dunyach-Remy C, Ngba Essebe C, Mesureur J, Sotto A, Pagès JM, Nicolas-Chanoine MH, Lavigne JP. Modulation of Membrane Influx and Efflux in Escherichia coli Sequence Type 131 Has an Impact on Bacterial Motility, Biofilm Formation, and Virulence in a Caenorhabditis elegans Model. Antimicrob Agents Chemother. 2016 Apr 22;60(5):2901-11. doi: 10.1128/AAC.02872-15.

255. Pradel E, Pagès JM. 2002. The AcrAB-TolC efflux pump contributes to multidrug resistance in the nosocomial pathogen *Enterobacter aerogenes.* Antimicrob Agents Chemother 46:2640-2643.

256. Pérez A, Canle D, Latasa C, Poza M, Beceiro A, Tomás Mdel M, Fernández A, Mallo S, Pérez S, Molina F, Villanueva R, Lasa I, Bou G. 2007. Cloning, nucleotide sequencing, and analysis of the AcrAB-TolC efflux pump of *Enterobacter cloacae* and determination of its involvement in antibiotic resistance in a clinical isolate. Antimicrob Agents Chemother 51:3247-3253.

257. Masi M, Pagès JM, Pradel E. 2003. Overexpression and purification of the three components of the *Enterobacter aerogenes* AcrA-AcrB-TolC multidrug efflux pump. J Chromatogr B Analyt Technol Biomed Life Sci 786:197-205.

258. Masi M, Saint N, Molle G, Pagès JM. 2007. The *Enterobacter aerogenes* outer membrane efflux proteins TolC and EefC have different channel properties. Biochim Biophys Acta 1768:2559-2567.

259. Li XZ, Plésiat P, Nikaido H. 2015. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. Clin Microbiol Rev 28:337-418. doi: 10.1128/CMR.00117-14.

260. Du D, Wang-Kan X, Neuberger A, van Veen HW, Pos KM, Piddock LJV, Luisi BF. 2018. Multidrug efflux pumps: structure, function and regulation. Nat Rev Microbiol 16:523-539. doi: 10.1038/s41579-018-0048-6.

261. Saier MH Jr, Reddy VS, Tamang DG, Västermark A. 2014. The transporter classification database. Nucleic Acids Res 42(Database issue):D251-8. doi: 10.1093/nar/gkt1097.

262. He GX, Thorpe C, Walsh D, Crow R, Chen H, Kumar S, Varela MF. 2011. EmmdR, a new member of the MATE family of multidrug transporters, extrudes quinolones from *Enterobacter cloacae*. Arch Microbiol 193:759-765. doi: 10.1007/s00203-011-0738-1.

263. He GX, Zhang C, Crow RR, Thorpe C, Chen H, Kumar S, Tsuchiya T, Varela MF. 2011. SugE, a new member of the SMR family of transporters, contributes to antimicrobial resistance in *Enterobacter cloacae*. Antimicrob Agents Chemother 55:3954-3957. doi: 10.1128/AAC.00094-11.

264. Schuldiner S. 2018. The *Escherichia coli* effluxome. Res Microbiol 169:357-362. doi: 10.1016/j.resmic.2018.02.006.

265. Neuberger A, Du D, Luisi BF. 2018. Structure and mechanism of bacterial tripartite efflux pumps. Res Microbiol 169:401-413. doi: 10.1016/j.resmic.2018.05.003.

266. Nikaido H, Pagès JM. 2012. Broad-specificity efflux pumps and their role in multidrug resistance of Gram-negative bacteria. FEMS Microbiol Rev 36:340-363. doi: 10.1111/j.1574-6976.2011.00290.x.

267. Guérin F, Isnard C, Sinel C, Morand P, Dhalluin A, Cattoir V, Giard JC. 2016. Cluster-dependent colistin hetero-resistance in *Enterobacter cloacae* complex. J Antimicrob Chemother 71:3058-3061.

268. Tran QT, Dupont M, Lavigne JP, Chevalier J, Pagès JM, Sotto A, Davin-Regli A. 2009. Occurrence of efflux mechanism and cephalosporinase variant in a population of *Enterobacter aerogenes* and *Klebsiella pneumoniae* isolates producing extended-spectrum beta-lactamases. Antimicrob Agents Chemother 53:1652-1656. doi: 10.1128/AAC.00822-08.

269. Du D, Wang Z, James NR, Voss JE, Klimont E, Ohene-Agyei T, Venter H, Chiu W, Luisi BF. 2014. Structure of the AcrAB-TolC multidrug efflux pump. Nature 509:512-515. doi: 10.1038/nature13205.

270. Hobbs EC, Yin X, Paul BJ, Astarita JL, Storz G. 2012. Conserved small protein associates with the multidrug efflux pump AcrB and differentially affects antibiotic resistance. Proc Natl Acad Sci U S A 109:16696-16701. doi: 10.1073/pnas.1210093109.

271. Wong MH, Chan EW, Chen S. 2015. Evolution and dissemination of OqxAB-like efflux pumps, an emerging quinolone resistance determinant among members of *Enterobacteriaceae*. Antimicrob Agents Chemother 59:3290-3297. doi: 10.1128/AAC.00310-15.

272. Andrade LN, Siqueira TES, Martinez R, Darini ALC. 2018. Multidrug-Resistant CTX-M-(15, 9, 2) and KPC-2-Producing *Enterobacter hormaechei* and *Enterobacter asburiae* isolates possessed a set of acquired heavy metal tolerance genes including a chromosomal *sil* operon (for acquired silver resistance). Front Microbiol 9:539. doi: 10.3389/fmicb.2018.00539.

273. Chollet R, Chevalier J, Bollet C, Pagès JM, Davin-Regli A. 2004. RamA is an alternate activator of the multidrug resistance cascade in *Enterobacter aerogenes*. Antimicrob Agents Chemother 48:2518-2523.

274. Cinquin B, Maigre L, Pinet E, Chevalier J, Stavenger RA, Mills S, Réfrégiers M, Pagès JM. 2015. Microspectrometric insights on the uptake of antibiotics at the single bacterial cell level. Sci Rep 5:17968. doi: 10.1038/srep17968.

275. Kaščáková S, Maigre L, Chevalier J, Réfrégiers M, Pagès JM. 2012. Antibiotic transport in resistant bacteria: synchrotron UV fluorescence microscopy to determine antibiotic accumulation with single cell resolution. PLoS One 7(6):e38624. doi: 10.1371/journal.pone.0038624.

276. Vergalli J, Dumont E, Cinquin B, Maigre L, Pajovic J, Bacqué E, Mourez M, Réfrégiers M, Pagès JM. 2017. Fluoroquinolone structure and translocation flux across bacterial membrane. Sci Rep 7:9821. doi: 10.1038/s41598-017-08775-4.

277. Vergalli J, Dumont E, Pajović J, Cinquin B, Maigre L, Masi M, Réfrégiers M, Pagés JM. 2018. Spectrofluorimetric quantification of antibiotic drug concentration in bacterial cells for the characterization of translocation across bacterial membranes. Nat Protoc 13:1348-1361. doi: 10.1038/nprot.2018.036. Epub 2018 May 17.

278. Ghisalberti D, Masi M, Pagès JM, Chevalier J. 2005. Chloramphenicol and expression of multidrug efflux pump in *Enterobacter aerogenes*. Biochem Biophys Res Commun 328:1113-1118.

279. Fisher RA, Gollan B, Helaine S. Persistent bacterial infections and persister cells. Nat Rev Microbiol. 2017 Aug;15(8):453-464. doi: 10.1038/nrmicro.2017.42.

280. Pérez A, Poza M, Aranda J, Latasa C, Medrano FJ, Tomás M, Romero A, Lasa I, Bou G. 2012. Effect of transcriptional activators SoxS, RobA, and RamA on expression of multidrug efflux pump AcrAB-TolC in *Enterobacter cloacae*. Antimicrob Agents Chemother 56:6256-6266. doi: 10.1128/AAC.01085-12.

281. Tzouvelekis LS, Tzelepi E, Kaufmann ME, Mentis AF. 1994. Consecutive mutations leading to the emergence in vivo of imipenem resistance in a clinical strain of *Enterobacter aerogenes*. J Med Microbiol 40:403-407.

282. Baron S, Bardet L, Dubourg G, Fichaux M, Rolain JM. 2017. *mcr*-1 plasmid-mediated colistin resistance gene detection in an *Enterobacter cloacae* clinical isolate in France. J Glob Antimicrob Resist 10:35-36. doi: 10.1016/j.jgar.2017.05.004.

283. Uechi K, Tada T, Shimada K, Nakasone I, Kirikae T, Fujita J. 2018. Emergence of a carbapenem-resistant and colistin-heteroresistant *Enterobacter cloacae* clinical isolate in Japan. J Infect Chemother Sep 29. pii: S1341-321X(18)30305-2. doi: 10.1016/j.jiac.2018.09.004.

284. Telke AA, Olaitan AO, Morand S, Rolain JM. 2017. SoxRS induces colistin hetero-resistance in *Enterobacter asburiae* and *Enterobacter cloacae* by regulating the acrAB-tolC efflux pump. J Antimicrob Chemother 72:2715-2721. doi: 10.1093/jac/dkx215.

285. Zeng KJ, Doi Y, Patil S, Huang X, Tian GB. 2016. Emergence of the Plasmid-Mediated *mcr-1* gene in colistin-resistant *Enterobacter aerogenes* and *Enterobacter cloacae*. Antimicrob Agents Chemother 60:3862-3863. doi: 10.1128/AAC.00345-16.

286. Prim N, Turbau M, Rivera A, Rodríguez-Navarro J, Coll P, Mirelis B. 2017. Prevalence of colistin resistance in clinical isolates of *Enterobacteriaceae*: A four-year cross-sectional study. J Infect 75:493-498. doi: 10.1016/j.jinf.2017.09.008.

287. Guérin F, Lallement C, Isnard C, Dhalluin A, Cattoir V, Giard JC. 2016. Landscape of resistance-nodulation-cell division (rnd)-type efflux pumps in *Enterobacter cloacae* complex. Antimicrob Agents Chemother 60:2373-2382. doi: 10.1128/AAC.02840-15.

288. Dautzenberg MJD, Bayjanov JR, Leverstein-van Hall MA, Muller AE, Gelinck LBS, Jansen CL, Leyten EMS, Ruys T, Scharringa J, van der Starre RE, Fluit AC, Bonten MJM. 2018. Dynamics of colistin and tobramycin resistance among *Enterobacter cloacae* during prolonged use of selective decontamination of the digestive tract. Antimicrob Resist Infect Control 7:67. doi: 10.1186/s13756-018-0356-7.

289. Kohlmann R, Bähr T, Gatermann SG. 2018. Species-specific mutation rates for *ampC* derepression in Enterobacterales with chromosomally encoded inducible AmpC β-lactamase. J Antimicrob Chemother 73:1530-1536. doi: 10.1093/jac/dky084.

290. Guérin F, Isnard C, Cattoir V, Giard JC. 2015. Complex regulation pathways of AmpC-mediated β-lactam resistance in *Enterobacter cloacae* complex. Antimicrob Agents Chemother 59:7753-7761. doi: 10.1128/AAC.01729-15.

291. Islam MA, Islam M, Hasan R, Hossain MI, Nabi A, Rahman M, Goessens WHF, Endtz HP, Boehm AB, Faruque SM. 2017. Environmental spread of new delhi metallo-β-lactamase-1-producing multidrug-resistant bacteria in Dhaka, Bangladesh. Appl Environ Microbiol 83. pii: e00793-17. doi: 10.1128/AEM.00793-17.

292. Livermore DM, Mushtaq S, Barker K, Hope R, Warner M, Woodford N. 2012. Characterization of β-lactamase and porin mutants of *Enterobacteriaceae* selected with ceftaroline + avibactam (NXL104). J Antimicrob Chemother 67:1354-8. doi: 10.1093/jac/dks079.

293. Deguchi T, Yasuda M, Nakano M, Ozeki S, Kanematsu E, Nishino Y, Ishihara S, Kawada Y. 1997. Detection of mutations in the *gyrA* and *parC* genes in quinolone-resistant clinical isolates of *Enterobacter cloacae*. J Antimicrob Chemother 40:543-549.

294. De Jong A, Muggeo A, El Garch F, Moyaert H, de Champs C, Guillard T. 2018. Characterization of quinolone resistance mechanisms in *Enterobacteriaceae* isolated from companion animals in Europe (ComPath II study). Vet Microbiol 216:159-167. doi: 10.1016/j.vetmic.2018.02.002.

295. Linde HJ, Notka F, Irtenkauf C, Decker J, Wild J, Niller HH, Heisig P, Lehn N. 2002. Increase in MICs of ciprofloxacin in vivo in two closely related clinical isolates of *Enterobacter cloacae*. J Antimicrob Chemother 49:625-630.

296. Nordmann P, Poirel L. 2005. Emergence of plasmid-mediated resistance to quinolones in *Enterobacteriaceae*. J Antimicrob Chemother 56:463-469.

297. Park YJ, Yu JK, Kim SI, Lee K, Arakawa Y. 2009. Accumulation of plasmid-mediated fluoroquinolone resistance genes, *qepA* and *qnrS1*, in *Enterobacter aerogenes* co-producing RmtB and class A beta-lactamase LAP-1. Ann Clin Lab Sci Winter 39:55-59.

298. Jlili Nel-H, Réjiba S, Smaoui H, Guillard T, Chau F, Kechrid A, Cambau E. 2014. Trend of plasmid-mediated quinolone resistance genes at the Children's Hospital in Tunisia. J Med Microbiol 63:195-202. doi: 10.1099/jmm.0.062216-0.

299. Osei Sekyere J, Amoako DG. 2017. Genomic and phenotypic characterisation of fluoroquinolone resistance mechanisms in *Enterobacteriaceae* in Durban, South Africa. PLoS One 12:e0178888. doi: 10.1371/journal.pone.0178888.

300. Albornoz E, Tijet N, De Belder D, Gomez S, Martino F, Corso A, Melano RG, Petroni A. 2017. *qnrE1*, a member of a new family of plasmid-located quinolone resistance genes, originated from the chromosome of *Enterobacter* species. Antimicrob Agents Chemother 61(5). pii: e02555-16. doi: 10.1128/AAC.02555-16.

301. Davin-Regli A, Bolla J-M, James CE, Lavigne J-P, Chevalier J, Garnotel E, Molitor A, Pagès JM. 2008. Membrane permeability and regulation of drug “influx and efflux” in enterobacterial pathogens. Curr Drug Targets 9: 750–759.

302. Cha MK, Kang CI, Park GE, Kim SH, Chung DR, Peck KR, Song JH. 2018 Genetic characterisation of tigecycline-resistant *Enterobacter* spp. in blood isolates causing bacteraemia. J Glob Antimicrob Resist 13:115-118. doi: 10.1016/j.jgar.2017.12.016.

303. Veleba M, Higgins PG, Gonzalez G, Seifert H, Schneiders T. 2012. Characterization of RarA, a novel AraC family multidrug resistance regulator in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 56:4450-4458. doi: 10.1128/AAC.00456-12.

304. Veleba M, De Majumdar S, Hornsey M, Woodford N, Schneiders T. 2013. Genetic characterization of tigecycline resistance in clinical isolates of *Enterobacter cloacae* and *Enterobacter aerogenes*. J Antimicrob Chemother 68:1011-1018. doi: 10.1093/jac/dks530.

305. De Florio L, Riva E, Giona A, Dedej E, Fogolari M, Cella E, Spoto S, Lai A, Zehender G, Ciccozzi M, Angeletti S. 2018. MALDI-TOF MS Identification and Clustering Applied to *Enterobacter* Species in Nosocomial Setting. Front Microbiol 9:1885. doi: 10.3389/fmicb.2018.01885.

306. Pavlovic M, Konrad R, Iwobi AN, Sing A, Busch U, Huber I. 2012. A dual approach employing MALDI-TOF MS and real-time PCR for fast species identification within the *Enterobacter cloacae* complex. FEMS Microbiol Lett 328:46–53.

307. Pham HN, Ohkusu K, Mishima N, Noda M, Monir Shah M, Sun X, Hayashi M, Ezaki T. 2007. Phylogeny and species identification of the family *Enterobacteriaceae* based on dnaJ sequences. Diagn Microbiol Infect Dis 58:153–161.

308. Mishra M, Patole S, Mohapatra H. 2017. Draft genome sequences of nonclinical and clinical *Enterobacter cloacae* isolates exhibiting multiple antibiotic resistance and virulence factors. Genome Announc 5(45). pii: e01218-17. doi: 10.1128/genomeA.01218-17.

309. Muresu R, Maddau G, Delogu G, Cappuccinelli P, Squartini A. 2010. Bacteria colonizing root nodules of wild legumes exhibit virulence-associated properties of mammalian pathogens. Antonie Van Leeuwenhoek 97:143-53. doi: 10.1007/s10482-009-9396-6.

310. Paton AW, Paton JC. 1996. *Enterobacter cloacae* producing a Shiga-like toxin II-related cytotoxin associated with a case of hemolytic-uremic syndrome. J Clin Microbiol 34:463–465.

311. Probert WS, McQuaid C, Schrader K. 2014. Isolation and identification of an *Enterobacter cloacae* strain producing a novel subtype of Shiga toxin type 1. J Clin Microbiol 52:2346–2351. doi:10.1128/JCM.00338-14.

312. Simi S, Carbonell GV, Falcón RM, Gatti MS, Joazeiro PP, Darini AL, Yano T. 2003. A low molecular weight enterotoxic hemolysin from clinical *Enterobacter cloacae.* Can J Microbiol 49:479-82.

313. Grazziotin AL, Vidal NM, Palmeiro JK, Dalla-Costa LM, Venancio TM. 2016. Genome sequencing of four multidrug-resistant *Enterobacter aerogenes* isolates from hospitalized patients in Brazil. Front Microbiol 7:1649. eCollection.

314. Lau YY, Yin WF, Chan KG. 2014. *Enterobacter asburiae* strain L1: complete genome and whole genome optical mapping analysis of a quorum sensing bacterium. Sensors (Basel) 14:13913-13924. doi: 10.3390/s140813913.

315. Liu F, Yang J, Xiao Y, Li L, Yang F, Jin Q. 2016. Complete Genome Sequence of a Clinical Isolate of *Enterobacter asburiae*. Genome Announc.4(3). pii: e00523-16. doi: 10.1128/genomeA.00523-16.

316. Ren Y, Ren Y, Zhou Z, Guo X, Li Y, Feng L, Wang L. 2010. Complete genome sequence of *Enterobacter cloacae* subsp. *cloacae* type strain ATCC 13047. J Bacteriol 192:2463-2464. doi: 10.1128/JB.00067-10.

317. Brooks LE, Ul-Hasan S, Chan BK, Sistrom MJ. 2018. Quantifying the evolutionary conservation of genes encoding multidrug efflux pumps in the ESKAPE pathogens to identify antimicrobial drug targets. mSystems 3. pii: e00024-18. doi: 10.1128/mSystems.00024-18.

318. Ho PL, Shek RH, Chow KH, Duan RS, Mak GC, Lai EL, Yam WC, Tsang KW, Lai WM. 2005. Detection and characterization of extended-spectrum beta-lactamases among bloodstream isolates of Enterobacter spp. in Hong Kong, 2000-2002. J Antimicrob Chemother 55:326-32. Epub 2005 Jan 28.

319. Andrade LN, Siqueira TES, Martinez R, Darini ALC. 2018. Multidrug-Resistant CTX-M-(15, 9, 2) and KPC-2-Producing *Enterobacter hormaechei* and *Enterobacter asburiae* isolates possessed a set of acquired heavy metal tolerance genes including a chromosomal *sil* operon (for acquired silver resistance). Front Microbiol 9:539. doi: 10.3389/fmicb.2018.00539.

320. Bratu S, Landman D, Alam M, Tolentino E, Quale J. 2005. Detection of KPC carbapenem-hydrolyzing enzymes in *Enterobacter* spp. from Brooklyn, New York. Antimicrob Agents Chemother 49:776-778.

321. Yang B, Feng Y, McNally A, Zong Z. 2018. Occurrence of *Enterobacter hormaechei* carrying bla_{NDM-1} and bla_{KPC-2} in China. Diagn Microbiol Infect Dis 90:139-142.

322. Naas T, Cattoen C, Bernusset S, Cuzon G, Nordmann P. 2012. First identification of blaIMI-1 in an *Enterobacter cloacae* clinical isolate from France. Antimicrob Agents Chemother. 2012 56:1664-5. doi: 10.1128/AAC.06328-11.

323. Falcone M, Mezzatesta ML, Perilli M, Forcella C, Giordano A, Cafiso V, Amicosante G, Stefani S, Venditti M. 2009. Infections with VIM-1 metallo-{beta}-lactamase-producing Enterobacter cloacae and their correlation with clinical outcome. J Clin Microbiol. 47:3514-9. doi: 10.1128/JCM.01193-09.

324. Guillard T, Cholley P, Limelette A, Hocquet D, Matton L, Guyeux C, Lebreil AL, Bajolet O, Brasme L, Madoux J, Vernet-Garnier V, Barbe C, Bertrand X, de Champs O, on Behalf of CarbaFrEst Group C. 2015. Fluoroquinolone Resistance Mechanisms and population structure of *Enterobacter cloacae* non-susceptible to ertapenem in north-eastern France. Front Microbiol 23;6:1186. doi: 10.3389/fmicb.2015.01186.

325. Bogaerts P, Bouchahrouf W, de Castro RR, Deplano A, Berhin C, Piérard D, Denis O, Glupczynski Y. 2011. Emergence of NDM-1-producing *Enterobacteriaceae* in Belgium. Antimicrob Agents Chemother 55:3036-8. doi: 10.1128/AAC.00049-11. Epub 2011 Mar 28.

326. Ghosh R, Aggeler R. 1987. Effect of lipid fluidity upon the activity and structure of the 39 kDa porin from *Enterobacter cloacae* 908S. FEBS Lett 222:154-158.

327. Gibson JS, Cobbold RN, Heisig P, Sidjabat HE, Kyaw-Tanner MT, Trott DJ. 2010. Identification of Qnr and AAC(6')-1b-cr plasmid-mediated fluoroquinolone resistance determinants in multidrug-resistant *Enterobacter* spp. isolated from extraintestinal infections in companion animals. Vet Microbiol 143:329-336. doi: 10.1016/j.vetmic.2009.11.031.

328. Amin M, Mehdipour G, Navidifar T.2019. High distribution of 16S rRNA methylase genes rmtB and **armA** among *Enterobacter cloacae* strains isolated from an Ahvaz teaching hospital, Iran. Acta Microbiol Immunol Hung. 2019 Feb 21:1-12. doi: 10.1556/030.66.2019.009. [Epub ahead of print]

329. Keeney D, Ruzin A, Bradford PA. 2007. RamA, a transcriptional regulator, and AcrAB, an RND-type efflux pump, are associated with decreased susceptibility to tigecycline in *Enterobacter cloacae*. Microb Drug Resist 13:1-6.

330. Werts C, O'Callaghan D, Hofnung M, Charbit A.1993. Immunological relatedness of the LamB proteins among members of *Enterobacteriaceae*. J Gen Microbiol 139:881-887.

331. Masi M, Pagès JM, Villard C, Pradel E. 2005. The eefABC multidrug efflux pump operon is repressed by H-NS in *Enterobacter aerogenes*. J Bacteriol 187:3894-3897.

332. Masi M, Pagès JM, Pradel E. 2006. Production of the cryptic EefABC efflux pump in *Enterobacter aerogenes* chloramphenicol-resistant mutants. J Antimicrob Chemother 57:1223-1226.

AUTHORS BIO

Anne Davin-Regli is an associate professor of the Faculty of Pharmacy on the Medical Campus at Aix-Marseille University and a member of the team UMR_MD1 U1261 INSERM "Membranes and Therapeutic targets". She is a Hospital practitioner head of the unit of health and safety at the Aubagne hospital.

Her research interest focus on epidemiology of hospital acquired bacteria, antibiotic and biocide resistance of Gram negative bacteria, genetic regulation of the multiresistance in Enterobacteriaceae. She has over 50 international scientific papers. Among them 27 publications concern the genus *Enterobacter*

Jean-Philippe Lavigne is Medical Doctor and Professor of Microbiology in University Hospital of Nîmes and in the Faculty of Medicine Montpellier-Nîmes France since 2011.

He is the head of a team in INSERM U1047 « Bacterial virulence and infectious diseases » at University of Montpellier. He is the co-Director of the University Hospital Federation Chronic Infection (Aviesan) and of the French National Reference Centre of Brucellosis.

He studies the relation between the different mechanisms of resistance developed by bacteria and their virulence, the cooperation between these bacteria and the link betwen commensalism and pathogenicity. His work focuses on the model of chronic infections particularly the diabetic foot ulcers. He showed the adaptation of pathogenic bacteria in a stress environment encountered in these chronic wounds and the influence of efflux pumps and outer membrane porins in this adaptation. By the metagenomics, culturomics and metabolomics approaches, he proposed new concepts to differentiate colonization and infection. He has published 233 papers in international journals including Diabetes, Diabetes Care, Adv Funct Mater or Scientific Reports.

Jean-Marie Pagès, is Emeritus Research Director at INSERM (Institut National de la Santé et de la Recherche Médicale) and member of the research Unit "Membranes and Therapeutic targets", at the Medical Campus at Aix-Marseille University, Marseille France since 2017.

He was previously Chair of the UMR-MD1 "membrane Transporters, Chemoresistance and Drug Design" (Aix-Marseille Univ-Health Dept of French Army) from 2008 to 2017.

During this period, he studies the bacterial envelope permeability, from genetic regulation, biological activities to clinical aspects. His work focuses on the outer membrane porins and the drug efflux pumps and their key role in bacterial adaptation. Recently, as chair of a working group in IMI-Translocation consortium, he developed new methods to dissect the drug translocation across membranes of gram-negative bacteria and proposed new concepts to understand the molecular bases of antibiotic resistance. As member of the "Scientific Committee on Emerging and Newly Identified Health Risks", EC-DG SANCO, Bruxelles (2004-2010), he has contributed to several published scientific opinions and he is member of several Scientific Committees of European Institutes. He has published over 300 papers and abstracts in international journals including Nature Microbiology, PNAS, Nature Reviews Microbiology, EMBO J., and has presented his work and concepts during the main top International meetings (Gordon Research Conferences, Nature Conferences, ECCMID, ICAAC, etc.).