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

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Running title: Enterobacter spp. Epidemiology and Resistance

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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 (β-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).


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, norfloxacain, ciprofloxacain 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 producing 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 cloaceae 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,
cefpirrome 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
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 *blaCTX-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,
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 ludwigi* cohaboring 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 (*blaCTX-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 *Enterobacter* 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). *Enterobacter* 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 occurring 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. cloacae* 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, β-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,
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 β-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 β-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 (blaCMY -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 β-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 \textit{aac(6')-Ib} [named \textit{aac(6')-Ib-cr}], the aminoglycosides resistance enzymatic determinant, which became able to acetylate ciprofloxacin and norfloxacin (70, 223). The association with the \textit{blaOXA-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 \(\beta\)-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 \textit{E. aerogenes} (226, 227). Today, three general (nonspecific) porins have been identified in \textit{Enterobacter} spp, Omp35, Omp36 and Omp37, and, two additional specific porins, LamB and PhoE, have been identified and exhibit some similarities with \textit{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 \textit{E. aerogenes} isolates, which become less virulent in a \textit{Caenorhabditis elegans} model (94). This adverse effect of resistance mechanisms on \textit{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 \textit{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 \textit{E. aerogenes} and \textit{E. cloacae} (253-255). In addition, \textit{E. aerogenes} TolC and EefC have been documented and present different channel properties determined by using electrophysiology assays (256).

\textbf{iii) Efflux pumps and antibiotic activity}

Multidrug efflux pumps present on the \textit{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 \textit{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 *phoQ* and *pmrB*, 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 *pmrA* 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 found 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 which employs sequencing of four to seven of housekeeping genes such as *atpD, fusA, idC, infB, gyrB, leuS, dnaA, pyrG, rplB, rpoB* and *hsp60*, 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 recent 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 *cloaca* 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. cloaca* 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

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ABBREVIATIONS

AAC: aminoside-N-acétyltransférase

APH: aminoside-O-phosphotransférase

AAD: Aminosides adénylyltransférase

ANT: aminoside-O-nucléotidytransférase

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).

<table>
<thead>
<tr>
<th></th>
<th>Yellow Pigment</th>
<th>LDC</th>
<th>ADH</th>
<th>URE</th>
<th>ESC</th>
<th>Fermentations</th>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>INO</td>
</tr>
<tr>
<td><em>E. amnigenus</em> biogr. 1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>E. amnigenus</em> biogr. 2</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>E. cancerogenus</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>E. asburiae</em></td>
<td>-</td>
<td>(-)</td>
<td>4/-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>E. cloacae</em> subsp.</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>(-)</td>
</tr>
<tr>
<td><em>E. cloacae</em> subsp.</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+w</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td><em>E. hormaechei</em> subsp.</td>
<td>-</td>
<td>-</td>
<td>(+)</td>
<td>(+)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>E. kobei</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>E. ludwigii</em></td>
<td>-</td>
<td>(-)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>E. nimipressuralis</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>E. aerogenes</em></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>E. gergoviae</em></td>
<td>-</td>
<td>(+)</td>
<td>-</td>
<td>+w</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>E. mori</em></td>
<td>+</td>
<td>+</td>
<td>nd</td>
<td>nd</td>
<td>+</td>
<td>nd</td>
</tr>
<tr>
<td><em>E. bugandensis</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>w</td>
<td>+</td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>Antibiotics</th>
<th>Mechanisms of resistance</th>
<th>Genes</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactams</td>
<td>Enzymatic</td>
<td><strong>blaTEM, blaSHV, blaCTX-M</strong></td>
<td><em>E. cloacae,</em></td>
<td>(63, 318)</td>
</tr>
<tr>
<td>β-lactamases</td>
<td></td>
<td></td>
<td><em>E. hormaechei</em></td>
<td>(319)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>blaVEB, blaGES/IBC, blaKPC, blaFRI</strong></td>
<td><em>E. cloacae,</em></td>
<td>(212, 320)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>E. hormaechei</em></td>
<td>(321)</td>
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<td></td>
<td></td>
<td><strong>blaNMCA, blaIMI</strong></td>
<td><em>E. cloacae,</em></td>
<td>(322)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>E. asburiae</em></td>
<td>(54)</td>
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<td><strong>blaVIM, blaGIM</strong></td>
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<td><em>E. cloacae,</em></td>
<td>(69, 323, 324)</td>
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<td></td>
<td><strong>blaNDM</strong></td>
<td><em>E. ludwigii</em></td>
<td></td>
<td>(84)</td>
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<tr>
<td></td>
<td><strong>blaIMP, blaNDM</strong></td>
<td><em>E. cloacae</em></td>
<td></td>
<td>(211, 325)</td>
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<td>Class C</td>
<td><strong>ampC</strong></td>
<td><em>E. cloacae,</em></td>
<td><em>E. asburiae,</em></td>
<td>(290, 297)</td>
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<td></td>
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<td></td>
<td><em>E. hormaechei,</em></td>
<td>(3)</td>
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<td></td>
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<td></td>
<td><em>E. kobei</em></td>
<td>(215)</td>
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<td></td>
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<td></td>
<td><em>E. ludwigii</em></td>
<td>(165)</td>
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<td><em>E. nimpressuralis</em></td>
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<td>Class D</td>
<td><strong>blaOXA-48</strong></td>
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<td></td>
<td>Impermeability/efflux</td>
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<tr>
<td>Fluoroquinolones</td>
<td>Target mutation</td>
<td><strong>gyrA, gyr B, parC, parE</strong></td>
<td><em>E. cloacae</em></td>
<td>(293)</td>
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<td>Enzymatic (acetyltransferase)</td>
<td><strong>aac(6’)-Ib-cr</strong></td>
<td><em>E. cloacae</em></td>
<td>(150)</td>
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<td>Protective mechanism target</td>
<td><strong>qnr</strong> (A, B, S, C, D)</td>
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<td>(327)</td>
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<td><em>E. hormaechei</em></td>
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<td></td>
<td>Efflux</td>
<td><strong>qepA, acrAB-tolC, oqxAB, sugE, emmdR</strong></td>
<td><em>E. cloacae</em></td>
<td>(262, 263, 267)</td>
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<td>Aminoglycosides</td>
<td>Enzymatic (acetyl, phospho, nucleotidyltransferase)</td>
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<td>Methylase</td>
<td><strong>armA, rmtB</strong></td>
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<td>Cyclines</td>
<td>Efflux</td>
<td><strong>acrAB-tolC</strong></td>
<td><em>E. cloacae</em></td>
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<td></td>
<td></td>
<td></td>
<td><em>E. hormaechei</em></td>
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TABLE 3

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

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Name/protein</th>
<th>Function</th>
<th>characteristics</th>
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<td></td>
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<tr>
<td></td>
<td>OmpE35</td>
<td>general porin</td>
<td>trimer</td>
<td>(232, 235)</td>
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<tr>
<td></td>
<td>OmpE36</td>
<td>general porin</td>
<td>trimer</td>
<td>(232, 235)</td>
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<td></td>
<td>OmpE37</td>
<td>quiescent porin</td>
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<td>OmpX</td>
<td></td>
<td>monomer</td>
<td>(242)</td>
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<tr>
<td></td>
<td>OmpA</td>
<td>OM architecture</td>
<td>nd</td>
<td></td>
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<tr>
<td></td>
<td>LamB</td>
<td></td>
<td></td>
<td>(330)</td>
</tr>
<tr>
<td></td>
<td>PhoE</td>
<td></td>
<td></td>
<td>(244)</td>
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<td></td>
<td>EmmdR</td>
<td>IM transporter</td>
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<td>(262, 263)</td>
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<td></td>
<td>SugE</td>
<td>IM transporter</td>
<td></td>
<td>(262, 263)</td>
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<td></td>
<td>TolC</td>
<td>OM channel</td>
<td>trimer</td>
<td>(256)</td>
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<td>AcrA</td>
<td>adapter</td>
<td>nd</td>
<td>(256)</td>
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<tr>
<td></td>
<td>AcrB</td>
<td>IM transporter</td>
<td>trimer</td>
<td>(256)</td>
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<td>Omp35</td>
<td>general porin</td>
<td>trimer</td>
<td>(232, 245)</td>
</tr>
<tr>
<td></td>
<td>Omp36</td>
<td>general porin</td>
<td>trimer</td>
<td>(232, 245)</td>
</tr>
<tr>
<td></td>
<td>Omp37</td>
<td>quiescent porin</td>
<td>trimer</td>
<td>Bornet unpublished data</td>
</tr>
<tr>
<td></td>
<td>OmpX</td>
<td>small channel</td>
<td>nd</td>
<td>(241)</td>
</tr>
<tr>
<td></td>
<td>OmpA</td>
<td>OM architecture</td>
<td>monomer</td>
<td>(201, 226)</td>
</tr>
<tr>
<td></td>
<td>LamB</td>
<td>maltoporin</td>
<td>trimer</td>
<td>(240)</td>
</tr>
<tr>
<td></td>
<td>PhoE</td>
<td>phosphoporin</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>Tsx</td>
<td>nucleoside uptake</td>
<td>nd</td>
<td>(227)</td>
</tr>
<tr>
<td></td>
<td>TolC</td>
<td>OM channel</td>
<td>trimer</td>
<td>(255)</td>
</tr>
<tr>
<td></td>
<td>AcrA</td>
<td>adapter</td>
<td>nd</td>
<td>(255)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>---</td>
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<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>AcrB</td>
<td>IM transporter</td>
<td>trimer</td>
<td>(255)</td>
<td></td>
</tr>
<tr>
<td>EefC</td>
<td>OM channel</td>
<td>trimer</td>
<td>(331, 332)</td>
<td></td>
</tr>
<tr>
<td>EefA</td>
<td>adapter</td>
<td>nd</td>
<td>(331, 332)</td>
<td></td>
</tr>
<tr>
<td>EefB</td>
<td>IM transporter</td>
<td>trimer</td>
<td>(331, 332)</td>
<td></td>
</tr>
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</table>
**TABLE 4**

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

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster I</td>
<td><em>E. asburiae</em></td>
</tr>
<tr>
<td>Cluster II</td>
<td><em>E. kobei</em></td>
</tr>
<tr>
<td>Cluster III</td>
<td><em>E. hormaechei</em> subsp. <em>hoffmannii</em></td>
</tr>
<tr>
<td>Cluster IV</td>
<td><em>E. toggenkampii</em></td>
</tr>
<tr>
<td>Cluster V</td>
<td><em>E. ludwigii</em></td>
</tr>
<tr>
<td>Cluster VI</td>
<td><em>E. hormaechei</em> subsp. <em>oharae and xiangfangensis</em></td>
</tr>
<tr>
<td>Cluster VII</td>
<td><em>E. hormaechei</em> subsp. <em>hormaechei</em></td>
</tr>
<tr>
<td>Cluster VIII</td>
<td><em>E. hormaechei</em> subsp. <em>steigerwalti</em></td>
</tr>
<tr>
<td>Cluster IX</td>
<td><em>E. bugandensis</em></td>
</tr>
<tr>
<td>Cluster X</td>
<td><em>E. nimipressuralis</em></td>
</tr>
<tr>
<td>Cluster XI</td>
<td><em>E. cloacae</em> subsp. <em>cloacae</em></td>
</tr>
<tr>
<td>Cluster XII</td>
<td><em>E. cloacae</em> subsp. <em>dissolvens</em></td>
</tr>
<tr>
<td>Cluster XIII</td>
<td><em>E. cloacae</em> sequence crowd</td>
</tr>
</tbody>
</table>
REFERENCES


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.

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He studies the relation between the different mechanisms of resistance developed by bacteria and their virulence, the cooperation between these bacteria and the link between 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.

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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.).