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Clinical and pathogenesis overview of Enterobacter infections

Kobra Salimiyan Rizi (Ph.D Candidate)[1], Kiarash Ghazvini (Ph.D)[2], Hadi Farsiani* (Ph.D)[2]

[1] *Department of Microbiology and Virology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
[2] *Antimicrobial Resistance Research Center, Department of Microbiology and Virology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*

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ABSTRACT

Enterobacter spp. is a gram-negative environmental bacterium, which belongs to the Enterobacteriaceae family and is found in water, sewage, soil, and plants. These bacteria are common among humans and animals, and the most frequently isolated species is Enterobacter cloacae. The species of this genus are often opportunistic pathogens with expanding significance in nosocomial infections, particularly in neonates, immunocompromised patients in intensive care units, emergency sections, skin and soft tissue infection wards, and urology wards. With the unexpected and rapid increase in antibiotic resistance in various bacterial species, there has been a new alarm for the health of the human community. Enterobacter species cause pneumonitis, bacteremia, post-neurosurgical meningitis, neonatal meningitis, skin and soft tissue infections, and urinary tract infections. Some of the main risk factors for the occurrence and dissemination of Enterobacter spp. infections are poor hand hygiene, crowding, low birth weight, premature birth, intubation of patients, prolonged hospital stay, contaminated infant formula, intravenous feeding, use of extended-spectrum antibiotics and use of intravenous catheters.

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Literature Review

Enterobacter is a fermentative gram-negative bacterium, which belongs to the Enterobacteriaceae family. This genus was introduced by Hormaeche and Edwards (1960a) (1). The ability of the genus Enterobacter spp. to survive in a wide range of environmental conditions leads to the outbreak of various infections in medical settings (2). These pathogens are mostly members of the gastrointestinal microbiota and respiratory tract of humans and animals (3, 4). These bacteria are facultative, anaerobic rod-shaped, and motile by peritrichous flagella (4-6 in general), and their glucose fermentation occurs with acid and gas production.

Most Enterobacteriaceae strains have been reported to have a positive Voges-Proskauer reaction and a negative methyl red test. The reduction of nitrate to nitrite and alkaline reaction in Simmons citrate and malonate broth are also positive. However, no selective media is available for Enterobacter species (5). Normally, they are associated with the contaminations caused by intravenous injection fluids, blood products, stethoscope, cotton swabs, and colonized hands of healthcare professionals (6).

Recently and mainly in the past decade, infectious episodes due to the resistance of several gram-negative bacteria (e.g., Enterobacter spp.) have been observed in various regions in the world (7, 8). An insignificant bacterium could threaten the health of the human community. Therefore,

Corresponding author: Hadi Farsiani.
Antimicrobial Resistance Research Center, Department of Microbiology and Virology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
E-mail: *farsianih@mums.ac.ir*
Tel:+ *989127853225*

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it is essential to acquire more knowledge regarding this bacterium and review its taxonomy classification and properties. In this genus, classification was described after delineation via DNA-DNA hybridizations. To date, 22 species have been detected in this genus. Some of the more clinically significant species that have been identified recently or in the past in the genus Enterobacter include Enterobacter cloacae, Klebsiella aerogenes (E. aerogenes), E. amnigenus, Cronobacter sakazakii (E. sakazakii), E. gergoviae, E. intermedium, Pantoea agglomerans (E. agglomerans), E. cancerogenus (E. taylorae), E. asburiae, E. hormaechei, and E. dissolvens (1, 9). Enterobacter spp. strains differ with each other in molecular methods, in which the 16SrRNA gene, oriC locus, and gyrB are used as targets. The sequencing of a fragment of hsp60 is considered to be a discriminator tool with 12 genomic clusters that are propagated by Hoffmann and Roggenkamp clusters I-XII. In 16SrRNA analysis, Enterobacter has been arranged as a polyphyletic genus, and most of the species could not be determined (10). Furthermore, the typing method has been applied to determine the source and modes of transmission in the epidemic strains. Some of the approaches in this regard include biotyping, serotyping, phage typing, bacteriocin typing, plasmid profile, ribotyping, pulse-field gel electrophoresis, and multi-locus enzyme electrophoresis testing. Polymerase chain reaction (PCR)-based methods such as repetitive extragenic palindromic PCR and enterobacterial repetitive intergenic consensus PCR are considered to be effective methods for the detection of epidemic circulating strains during outbreaks on a day-to-day basis (11, 12).

This review aimed to introduce several important species of this bacterium from a clinical perspective as they are involved in hazardous human infections. Within a genetic complex group referred to as the Enterobacter cloacae complex, six genetically relevant and phenotypically similar species were intermixed, including Enterobacter cloacae, E. asburiae, E. dissolvens, E. hormaechei, E. kobei, and E. nimipressuralis. Several of these strains subscribe to a DNA fragment to affiliate with E. cloacae within the range of 61-67% (10,13). Furthermore, at least 12 genetic clusters have been phylogenetically defined within the complex (14, 15). Clinical bacteriologists have recognized E. cloacae based on the biochemical attributes, demonstrating a large complex of at least 13 diverse species, subspecies, and genotypes (15, 16). These clusters are often recognized as E. cloacae using commercial biochemical kits, such as API20E. It is also notable that matrix-assisted laser desorption ionization-time of flight mass spectrometry and E. cloacae-specific duplex real-time PCR in combination are used to identify E. cloacae complex species (17). Among these members, E. hormaechei and E. cloacae are often isolated from human clinical specimens, especially from the patients admitted at intensive care units (ICUs). Therefore, E. cloacae have been one of the most usual Enterobacter spp. strains causing nosocomial infections in the past decade, and there have been numerous publications on the antibiotic resistance properties of these bacteria. Moreover, it has been remarked as an opportunistic pathogen involved in a wide array of infections, while the exact mechanism of pathogenicity remains unclear (18, 19). This bacterium could form biofilms and contribute to the secretion of multiple cellular toxins, such as enterotoxins, hemolysins, and pore-forming toxins (10, 20, 21). Type III secretion system and curli fimbriae may contribute to its pathogenesis as well (22). The pathogenic strategies and agents that are involved in the diseases associated with the E. cloacae complex are not yet acknowledged, which could be due to the deficiency in the distribution of the available data in this regard [20]. The Enterobacter cloacae complex is an opportunistic pathogen, which causes infections in wounds, the urinary tract, musculoskeletal (postoperatively), bloodstream, skin and soft tissues, respiratory tract, and postsurgical peritonitis (23). In addition, it may occasionally cause blood and brain infections, especially in immunocompromised persons (24, 25). Drastic outbreaks of nosocomial and community infections are mostly caused by E. cloacae, E. asburiae, and E. hormaechei (10). E. amnigenus and E. asburiae strains have been isolated from human infections (4). Table 1 shows a summary of the biochemical properties of the important clinical Enterobacter species. Intrinsic resistance to aminopenicillins, amoxicillin-clavulanate, first-generation cephalosporins, and cefoxitin due to the production of constitutive AmpC β-lactamase (26). Aminoglycoside and carbapenem resistance have also been reported in this bacterium (10). The frequency of the clinical isolates of Enterobacter spp. producing extended-spectrum beta-lactamases (ESBL) highly varies in different countries. For instance, the rate has been estimated at 52.1% in Nigeria (27), 9% in Iran (28), 14.93% in Pakistan (29), 37.9% in France (30), 21.5 % in Taiwan (31), 6.7% in Barcelona (32), 7.6% in the Netherlands (33), and 17.7% in Algeria (34). ESBL productionin Enterobacter spp.

Table1. Characteristics of the clinical common species of the genus *Enterobacter*.

	Test	*E.cloacae* complex[b]	*K.aerogenes*	*C.sakazakii*	*P.agglomerans*		Test	*E.cloacae* complex[b]	*K.aerogenes*	*C.*	
1	Indole	-	-	D		20	Adonitol	d	+	-	d
2	Methyl Red	d	-	-	d	21	Raffinose	+	+	+	d
3	Voges-proskauer	d	+	+	d	22	L-Rhamnose	d	+	+	d
4	Citrate	+	+	+	+	23	Malonate	d	d	+	-
5	Urease	+ (65%)	-	-	-	24	Maltose	+	+	+	+
6	H_2S Production	-	-	-	-	25	D-glucose gas production	+	+	+	-
7	Oxidase	-	-	-	-	26	ONPG	+	+	+	+
8	Motility(36 °C)	d	+	+	+	27	Trehalose	+	+	+	+
9	Lysine decarboxylase	-	+	-	-	28	Glycerol	d	+	-	+
10	Ornithine decarboxylase	+	+	+	-	29	D-mannitol	+	+	+	+
11	Arginine dihydrolase	+	-	+	-	30	Dulcitol	D	d	-	-
12	Esculin hydrolysis	d	+	+	d	31	Catalase production	+	+	+	+
13	Sucrose	+	+	+	d	32	Deoxy ribonuclease (25 °C)	-	-	(+)	+
14	Melibiose	d	+	+	d	33	Lipase	-	+	-	d
15	D-sorbitol	+	+	-	d	34	Growth in KCN	+	+	+	+
16	Mucate	d	+	-	d	35	Phenylalanine deaminase	-	-	d	+
17	Lactose	d	+	+	d	36	Nitrate reductase	+	+	+	+
18	Gelatine hydrolysis (22°C)	(d)	+	-	+						
19	Salicin	D		+	d						

[a] +: 90–100% strains positive in 1–2 days, (+): 90–100% strains positive in 1–4 days, d: positive or negative in 1–4 days, D: test used to differentiate species within a complex, (d): positive or negative in 3–4 days.

[b] The *E. cloacae* complex includes *E. cloacae, E. dissolvens, E. hormaechei*, and *E. asburiae* (1, 78, 79).

(especially E. cloacae) must be differentiated from a constitutive AmpC hyperproduction phenotype, which is the most frequent mechanism of broad-spectrum cephalosporin resistance [35]. Among the ESBL-producing clinical isolates of Enterobacter spp., the CTX-M-1 group enzymes are highly prevalent (36, 37).

The crisis of carbapenem-resistant Enterobacteriaceae (CRE) has become a major public health concern worldwide. Carbapenemase enzymes are the major mechanism of carbapenem resistance in this family, while Klebsiella pneumoniae and Escherichia coli primarily represent the etiology of CRE infections, and carbapenem-resistant E. cloacae has occasionally been described due to its unique properties (38, 39).

New Delhi metallo-beta-lactamase (NDM)-producing E. cloacae was reported in various regions worldwide (39, 40). Fosfomycin is a bactericidal antibiotic that inhibits peptidoglycan synthesis, which has been used as a therapeutic solution for the management of invasive infections, including the infections caused by CRE strains (e.g., NDM-producing E. cloacae) (41). Moreover, VEB-producing Enterobacteriaceae (e.g., E. cloacae) are mostly found in Vietnam and Thailand (10). To date, tigecycline and colistin resistance has rarely been reported in the E. cloacae complex, which may indicate that effective therapeutic options against multidrug-resistant Enterobacter spp. (42-44). It is also notable that for many species of this genus, there have been no commercially available vaccines. It was originally known as Aerobacter aerogenes, while based on the most recent taxonomic modifications, the name was changed to Klebsiella aerogenes (45).Motility and urease activity tests have been reported to be negative, while ornithine decarboxylase activity have been shown to be positive for this bacterium (46). Enterobacter aerogenes and E. cloacae are the most frequent human pathogens in genus Enterobacter (47).

Klebsiella aerogenes has been recognized as an opportunistic pathogen appearing as a nosocomial pathogen in patients admitted to the ICU (20). K. aerogenes is often disseminated through cross-contamination due to surgery or constant therapy in hospitals in the patients using catheters. Moreover, K. aerogenes may mostly cause wound, respiratory, urinary tract, skin and soft tissues, gastrointestinal tract, and septic infections, as well as the fulminant form of necrotizing meningitis in infants (48-51). Lysine decarboxylase and arginine decarboxylase activity tests are used for the biochemical differentiation between E. cloacae and K. aerogenes, in which K. aerogenes (unlike E. cloacae) has positive and negative reactions, respectively (52). The clinical significance of this bacterium is largely due to its high antibiotic resistance rather than the self-bacterial virulence. It has been progressively introduced for resistance against various antimicrobials, leading to the appearance of multidrug-resistant clinical strains (53). Inherent resistance against aminopenicillins among the recent isolates of K. aerogenes has frequently displayed resistance against β-lactams antibiotics, and enzymatic degradation by plasmid-mediated, broad-spectrum β-lactamases has been recognized as the most common resistance mechanism (54).

The unnecessary use of the extended-spectrum cephalosporins and carbapenems in the antibiotic therapy of Enterobacter spp. infections leads to the emergence of pan-drug-resistant K. aerogenes isolates with resistance to carbapenems and colistin, for which no therapeutic selection has been accessible. The most effective approach to tackle K. aerogenes might involve proactive prevention practices. The adaptive evolution in K. aerogenes is mostly due to porin mutations and the efflux mechanism (55).

Cronobacter sakazakii was referred to as yellow-pigmented E. cloacae and determined as an inimitable species almost 40 years ago (56, 57). This bacterium was identified from E. cloacae based on biochemical test results (e.g., malonate utilization, sorbitol fermentation, urea hydrolysis, and presence of α-glucosidase), molecular methods (e.g., DNA-DNA hybridization and rRNA 16S sequencing), antibiotic susceptibility, and pigment production (58).

Among the five species of the Cronobacter genus (C. sakazakii, C. malonaticus, C. dublinensis, C. muytjensii, and C. universalis), C. sakazakii has more frequently been isolated from human infections (59, 60). However, C. sakazakii has been recovered from all age groups, while the most high-risk population has been reported to be neonates aged less than one year (61). This strain has been shown to cause bacteremia and meningitis first in infants and is associated with the use of powdered milk formula (47).

The isolation of C. sakazakii has also been accomplished from dairy products, infant foods, sausage meat, and raw herbal foods (62).

Cronobacter bacteria could survive in the food products that are not heated adequately or have not been pasteurized (63). In addition, contaminated powdered infant formula is considered to be the main source of C. sakazakii infections in infants (64). Recent reports have also highlighted the risk posed to immunocompromised adults, especially the elderly (65).

The infectious dose of Cronobacter spp. requires further investigation. In a study, Iversen and Forsythe primarily predicted that the infectious dose may be approximately 1,000 CFU (66). It has also been suggested that the infectious dose of Cronobacter spp. might be 10,000 CFU [65]. Cronobacter spp. have been clinically isolated from a wide range of samples, including the cerebrospinal fluid, bone marrow, blood, intestinal and respiratory tracts, urine, ear and eye swabs, and skin wounds (65).

Clinical personnel should be informed of the feasible risk of the infections caused by the utilization of nonsterile formula in neonatal healthcare settings. Necrotizing enterocolitis, neonatal sepsis, and meningitis are the most common manifestations of C. sakazakii infections in infants and children, with the mortality rate estimated at 40-80%, while diarrhea, urinary tract infection, and septicemia have also been reported in this regard. The manifestations in neonatal meningitis are often severe and include seizures, brain abscess, hydrocephalus, developmental delay, and death in 40-80% of the patients (67). Low-birth-weight and premature birth (neonates aged of 28 days) are presumed to be at the higher risk of infections compared to normal infants (62, 68). Until recently, C. sakazakii pathogenesis was unclear (56). Biofilm formation, production of capsular materials in some strains, surface endotoxin, specific bacterial adhesins, and siderophores to acquire iron and thermo-tolerance are among the other properties facilitating the survival and pathogenicity of this bacterial strain (62). In cell culture, it has been reported to exhibit clustered adhesion [56], while surviving in macrophages and penetrating diverse cells (e.g., endothelial cells) (69).

Several biochemical full automatic and semi-automatic systems have been endorsed to identify Cronobacter spp., including Api20E, ID32E, BIOLOG microarray, and Vitek 2 system, while they could only be used for possible identification with relatively low accuracy (43%) (70, 71). According to the protocol of the Food and Drug Administration (FDA), violet red bile glucose agar medium could be used for the isolation of Cronobacter spp. from food products (65). It is also notable that among various biochemical tests, 100% of C. sakazakii have been reported to be positive for α-glucose oxidase compared to 0% of other Enterobacter species although some other members of the Enterobacteriaceae family are also positive for this enzyme (72).

Diverse chromogenic agars that are based on the alpha-glucosidase possessed by Cronobacter spp. are available (69). Similar to C. sakazakii, E. hormaechei has been isolated from enteric feeding and other infant products (57, 73). The misidentification of E. hormaechei as C. sakazakii could lead to substantial consequences (74). The production of acid from raffinose occurs by Cronobacter spp., while E. hormaechei has no such function, and there is key biochemical difference between these strains in this regard (75). C. sakazakii is inherently resistant to all macrolides, lincomycin, clindamycin, streptogramins, rifampicin, fusidic acid, and fosfomycin. It is susceptible to numerous β-lactams, chloramphenicol, tetracyclines, aminoglycosides, antifolates, and quinolones (76).

Ampicillin-gentamicin and ampicillin-chloramphenicol have been traditionally used for the treatment of C. sakazakii infections. However, due to the emergence of resistance to ampicillin following the production of β-lactamase enzymes, it is recommended that combination therapy with carbapenems or newer cephalosporins be applied, along with a second agent (e.g., aminoglycosides) (77).

P. agglomerans is an environmental and agricultural bacterium of the genus Pantoea, which was reclassified into the genus from Enterobacter in 1989. The species of this genus include P. ananatis, P. citrea, P. dispersa, P. punctata, P. stewartii, and P. agglomerans, which are the type species of the genus Pantoea. All Pantoea species could be isolated from food, water, soil, and plants, where they may either be harmful or commensals (80, 81). Furthermore, it could be found among the normal hand flora in humans (82). The properties of this strain could be identified based on the produced yellow pigment on blood agar, as well as oxidase- and urease-negative and negative H2S. P. agglomerans is the most commonly isolated species in humans, which is often a causative factor for occupational diseases and human infections (81). This opportunistic human pathogen causes human infections, especially in children. Moreover, it is a potential candidate as a powdered infant milk formula-borne opportunistic pathogen. Nosocomial infections may also occur due to the contamination of medical equipment and fluids (80, 83, 84).

P. agglomerans could be obtained from both community and healthcare settings. However, no actual evidence has identified a separate evolutionary connection between plant-associated and clinical P. agglomerans isolates (85). P. agglomerans may cause soft tissue or bone/joint infections following penetrating trauma through vegetation, spondylodiscitis, tibial osteitis, bloodstream infections, urinary tract infection, pneumonia, and wound infections (80, 82).

According to the findings of Gonçalves et al., P. agglomerans is a permanent constituent of the flora in chronic periodontitis lesions, posing the risk of generalized infections, especially in immunocompromised and hospitalized hosts (75). The lipopolysaccharide from E. agglomerans is often found in cotton dust and has a high affinity for binding to the pulmonary surfactant (lipid-proteinaceous underlying matters), thereby converting its surface tension traits. Binding in the lungs may change the physiological properties of surfactant, acting as a feasible mechanism for pathogenesis by sinuses, which is a professional respiratory untidiness caused by the inhalation of cotton dust (86). P. agglomerans strains could also express extended-spectrum β-lactamase and carbapenemases, which highlights the clinical importance of this bacterium. However, the extensive majority of Enterobacter infections are the result of E. cloacae, E. aerogenes, and E. agglomerans, and other species may occasionally occur in distinct human infections.

Enterobacter cancerogenus (formerly E. taylorae) is another species that has rarely been isolated from human infections. It is more associated with wound infections and osteomyelitis and has intrinsic resistance to aminopenicillins (i.e., amoxicillin and amoxicillin-clavulanic acid) and/or cephalosporins (87, 88). The E. cloacae complex has been shown to have 18 clades (A-R). Clade R (Hoffmann cluster IX) has recently been defined to be E. bugandensis (3) as identified from E. cloacae based on biochemical tests (e.g., D-arabinose fermentation and ornithine decarboxylase test) (88). In addition, E. bugandensis has recently been described as a species of Enterobacter and is highly pathogenic in this genus (3). It could cause neonatal sepsis and other infections in infants, as well as severe infections in immunocompromised patients (3, 89).

The type of strain EB-247 is resistant to ampicillin, amoxicillin/clavulanic acid, piperacillin-sulbactam, piperacillin-tazobactam, cefalotin, cefuroxime, cefuroxime-axetil, cefoxitin, cefpodoxime, cefotaxime, ceftazidime, gentamicin, tobramycin, ciprofloxacin, norfloxacin, tetracycline, and trimethoprim/sulfamethoxazole. However, it is sensitive to imipenem, meropenem, and nitrofurantoin [89]. Enterobacter dissolvens and E. nimipressuralis have only been isolated from the environment and not observed in clinical specimens (1).

Among various gram-negative pathogens that are emerging as nosocomial pathogens (e.g., P. aeruginosa, Klebsiella, and Enterobacter spp.) that refuge intrinsic (chromosomal) or acquired (plasmid) antimicrobial resistance determinants, Enterobacter spp. infections have gained significance, especially in recent years (90). A wide range of clinical syndromes have also been attributed to Enterobacter spp., the rate of which is on the rise in ICUs and neonatal ICUs, mainly affecting patients with prolonged hospital stay. Infection could be acquired from endogenous or environmental sources. Contaminated medications or distilled water in mechanical ventilators and solutions used for parenteral nutrition in neonatal units are common transition sources of Enterobacter in these wards. Colonization of Enterobacter spp. in severely ill patients could occur in the gastrointestinal tract and other human body organs, thereby easily transmitting among patients. Furthermore, the presence of a foreign device, low birth weight in neonates, prematurity, and immunocompromised patients are among the other risk factors for the acquisition of Enterobacter infections.

Some of the main infections in the human body include bacteremia, skin and soft tissue infections, respiratory tract infection, severe septic arthritis, osteomyelitis, urinary tract infection, bone and joint infections, gastrointestinal tract infection, central nervous system infections. Most of the findings regarding bacteremia are not noteworthy (range: 56-100%). In most series, E. cloacae (range: 46-91%) is followed by E. aerogenes (range: 9-43%) E. agglomerans, C. sakazakii, and the range of 14-53% has been reported for other bacteremia types, which may occur incidentally, involving Enterobacter spp. as a polymicrobial condition (11, 91).

In the United States, Enterobacter spp. have recently outstripped Klebsiella spp. to become the third most common cause of nosocomial respiratory tract infections (25). In lung transplant recipients, Enterobacter spp. have also been recognized as the most frequent pathogens (11).

Conclusion

In general, immunization with the development of new vaccines and improved hygiene (particularly hand hygiene by hand washing in hospitals and care centers for patients and the elderly) are considered to the most common control strategies for nosocomial infections. Among gram-negative pathogens, the infections caused by Enterobacter spp. are emerging as nosocomial pathogens; such examples are P.aeruginosa and Klebsiella, which harbors intrinsic (chromosomal) or acquired (plasmid) antimicrobial resistance determinants and have gained significance, especially in recent years. E. cloacae, K. aerogenes, C. sakazakii, and P. agglomerans are often isolated from human

clinical specimens. Enterobacter infections include bacteremia, skin and soft tissue infections, respiratory tract infections, severe septic arthritis, osteomyelitis, urinary tract infection, bone and joint infections, gastrointestinal tract infection, and central nervous system infections. Some of the main predisposing factors for the acquisition of Enterobacter infections include the presence of a foreign device, low birth weight in neonates, premature birth, and immunocompromised patients due to any causes. Moreover, the unnecessary use of the extended-spectrum cephalosporins and carbapenems in the antibiotic treatment of Enterobacter spp. infections has led to the emergence of resistant strains among Enterobacter spp. The effective control strategies for nosocomial infections are immunization with the development of new vaccines and improved hygiene, particularly hand hygiene (e.g., hand washing in hospitals and care centers for patients and the elderly).

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Francine Grimont, Patrick A. D. Grimont. . The genus enterobacter. The Prokaryotes pp 197-214.
2. Chavda KD, Chen L1, Fouts DE, et al. Comprehensive genome analysis of carbapenemase-producing Enterobacter spp.: new insights into phylogeny, population structure, and resistance mechanisms. mBio. 2016;7. pii: e02093-16.
3. Pati NB, Doijad SP, Schultze T, et al. Enterobacter bugandensis: a novel enterobacterial species associated with severe clinical infection. Sci Rep. 2018;8:5392.
4. [Chart, H. Klebsiella, enterobacter, proteus and other enterobacteria: Pneumonia; urinary tract infection; opportunist infection. Medical Microbiology. Churchill Livingstone, 2012. 290-297.
5. William Barnaby Whitman; Bergey's Manual Trust, Bergey's manual of systematics of archaea and bacteria. [Hoboken, New Jersey] : Wiley, [2015] ©2015.
6. Dos Santos G, Solidonio E, Costa M, et al. Study of the Enterobacteriaceae group CESP (Citrobacter, Enterobacter, Serratia, Providencia, Morganella and Hafnia): a review. The Battle Against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs. 2015;2:794-805.
7. Boucher HW, Talbot GH, Bradley JS, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis. 2009;48:1-12.
8. Rice LB. Progress and challenges in implementing the research on ESKAPE pathogens. Infect Control Hosp Epidemiol. 2010;31:7-10.
9. McMullan R, Menon V, Beukers AG, et al. Cronobacter sakazakii infection from expressed breast milk, Australia. Emerg Infect Dis. 2018;24:393-394.
10. Mezzatesta ML, Gona F, Stefani S. Enterobacter cloacae complex: clinical impact and emerging antibiotic resistance. Future Microbiol. 2012;7:887-902.
11. Patel KK, Patel S. Enterobacter spp. An emerging nosocomial infection. International Journal of Applied Research 2016; 2: 532-538.
12. Stumpf AN, Roggenkamp A, Hoffmann H. Specificity of enterobacterial repetitive intergenic consensus and repetitive extragenic palindromic polymerase chain reaction for the detection of clonality within the Enterobacter cloacae complex. Diagn Microbiol Infect Dis. 2005;53:9-16.
13. Akbari M, Bakhshi B, Peerayeh SN. Particular distribution of Enterobacter cloacae strains isolated from urinary tract infection within clonal complexes. Iran Biomed J. 2016;20:49-55.
14. Hoffmann H, Roggenkamp A. Population genetics of the nomenspecies Enterobacter cloacae. Appl Environ Microbiol. 2003;69:5306-5318.
15. Hoffmann H, Stindl S, Ludwig W, et al. Reassignment of Enterobacter dissolvens to Enterobacter cloacae as E. cloacae subspecies dissolvens comb. nov. and emended description of Enterobacter asburiae and Enterobacter kobei. Syst Appl Microbiol. 2005;28:196-205.
16. Hoffmann H, Stindl S, Ludwig W, et al. Enterobacter hormaechei subsp. oharae subsp. nov., E. hormaechei subsp. hormaechei comb. nov., and E. hormaechei subsp. steigerwaltii subsp. nov., three new subspecies of clinical importance. J Clin Microbiol. 2005;43:3297-303.
17. Pavlovic M, Konrad R, Iwobi AN, et al. A dual approach employing MALDI-TOF MS and real-time PCR for fast species identification within the Enterobacter cloacae complex. FEMS Microbiol Lett. 2012;328:46-53.
18. Krzymińska S, Mokracka J, Koczura R, et al. Cytotoxic activity of Enterobacter cloacae human isolates. FEMS Immunol Med Microbiol. 2009;56:248-252.
19. Dalben M, Varkulja G, Basso M, et al. Investigation of an outbreak of Enterobacter cloacae in a neonatal unit and review of the literature. J Hosp Infect. 2008;70:7-14.
20. Davin-Regli A, Pagès JM. Enterobacter aerogenes and Enterobacter cloacae; versatile bacterial pathogens confronting antibiotic treatment. Front Microbiol. 2015;6:392.
21. Kim S-M, Lee H-W, Choi Y-W, et al. Involvement of curli fimbriae in the biofilm formation of Enterobacter cloacae. J Microbiol. 2012;50:175-178.
22. Stuber K, Frey J, Burnens AP, et al. Detection of type III secretion genes as a general indicator of bacterial virulence. Mol Cell Probes. 2003;17:25-32.
23. Ceroni D. Musculoskeletal Postoperative Infections due to Enterobacter cloacae complex: A New Reality?. J Med Microbiol Diagn, 2013;3:128.
24. Bonadio WA, Margolis D, Tovar M. Enterobacter cloacae bacteremia in children: a review of 30 cases in 12 years. Clin Pediatr (Phila). 1991;30:310-313.
25. [25] Jarvis WR, Martone WJ. Predominant pathogens in hospital infections. J Antimicrob Chemother. 1992;29 Suppl A:19-24.
26. Stock I, Grueger T, Wiedemann B. Natural antibiotic susceptibility of strains of the Enterobacter cloacae complex. Int J Antimicrob Agents. 2001;18:537-545.
27. Raji MA, Jamal W, Ojemeh O, et al. Sequence analysis of genes mediating extended-spectrum beta-lactamase (ESBL) production in isolates of Enterobacteriaceae in a Lagos Teaching Hospital, Nigeria. BMC Infect Dis. 2015;15:259.
28. Rizi KS, Peerayeh SN, Bakhshi B, et al. Prevalence of integrons and Antimicrobial Resistance Genes Among Clinical Isolates of Enterobacter spp. From Hospitals of Tehran. Int J Enteric Pathog. 2015;3: e22531.
29. Amin H, Zafar A, Ejaz H, et al. Phenotypic characterization of ESBL producing Enterobacter cloacae among children. Pak J Med Sci. 2013;29:144-147.
30. Robin F, Beyrouthy R, Bonacorsi S, et al. Inventory of extended-spectrum-β-lactamase-producing Enterobacteriaceae in France as assessed by a multicenter study. Antimicrob Agents Chemother. 2017;61. pii: e01911-16.
31. Lee C-C, Lee N-Y, Yan J-J, Lee H-C, Chen P-L, Chang C-M, et al. Bacteremia due to extended-spectrum-β-lactamase-producing Enterobacter cloacae: role of carbapenem therapy. Antimicrob Agents Chemother. 2010;54:3551-3556.
32. Manzur A, Tubau F, Pujol M, et al. Nosocomial outbreak due to extended-spectrum-beta-lactamase-producing Enterobacter cloacae in a cardiothoracic intensive care unit. J Clin Microbiol. 2007;45:2365-2369.
33. Willemsen I, Oome S, Verhulst C, et al. Trends in extended spectrum beta-lactamase (ESBL) producing Enterobacteriaceae and ESBL genes in a Dutch teaching hospital, measured in 5 yearly point prevalence surveys (2010-2014). PLoS One. 2015;10:e0141765 .
34. Iabadene H, Messai Y, Ammari H, et al. Dissemination of ESBL and Qnr determinants in Enterobacter cloacae in Algeria. J Antimicrob Chemother. 2008;62:133-136.

35. Bell J. SENTRY Asia-Pacific Participants: Prevalence of extended-spectrum β-lactamase-producing Enterobacter cloacae in the Asia-Pacific Region: results from the SENTRY antimicrobial surveillance program, 1998 to 2001. Antimicrob Agents Chemother. 2003;47:3989-3993.
36. Chapuis A, Amoureux L, Bador J, et al. Outbreak of extended-spectrum beta-lactamase producing Enterobacter cloacae with high MICs of quaternary ammonium compounds in a hematology ward associated with contaminated sinks. Front Microbiol. 2016;7:1070.
37. Khalaf NG, Eletreby MM, Hanson ND. Characterization of CTX-M ESBLs in Enterobacter cloacae, Escherichia coli and Klebsiella pneumoniae clinical isolates from Cairo, Egypt. BMC Infect Dis. 2009;9:84.
38. Alam MZ, Aqil F, Ahmad I, et al. Incidence and transferability of antibiotic resistance in the enteric bacteria isolated from hospital wastewater. Braz J Microbiol. 2014;44:799-806 .
39. Shi Z, Zhao H, Li G, et al. Molecular characteristics of carbapenem-resistant Enterobacter cloacae in Ningxia Province, Front Microbiol. 2017;8:94.
40. Ho HJ, Toh CY, Ang B, et al. Outbreak of New Delhi metallo-β-lactamase-1–producing Enterobacter cloacae in an acute care hospital general ward in Singapore. Am J Infect Control. 2016;44:177-182.
41. Michalopoulos AS, Livaditis IG, Gougoutas V. The revival of fosfomycin. Int J Infect Dis. 2011;15:e732-739.
42. Anthony KB, Fishman NO, Linkin DR, et al. Clinical and microbiological outcomes of serious infections with multidrug-resistant gram-negative organisms treated with tigecycline. Clin Infect Dis. 2008;46:567-570.
43. Falagas ME, Kasiakou SK, Saravolatz LD. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis. 2005;40:1333-1341.
44. Tascini C, Urbani L, Biancofiore G, Ret al. Colistin in combination with rifampin and imipenem for treating a blaVIM-1 metallo-beta-lactamase-producing Enterobacter cloacae disseminated infection in a liver transplant patient. Minerva Anestesiol. 2008;74:47-49.
45. Tindall B, Sutton G, Garrity G. Enterobacter aerogenes Hormaeche and Edwards 1960 and Klebsiella mobilis Bascomb et al. 1971 share the same nomenclatural type (ATCC 13048) on the Approved Lists and are homotypic synonyms, with consequences for the name Klebsiella mobilis Bascomb et al. 1971. Int J Syst Evol Microbiol. 2017;67:502-504.
46. Farmer J, Davis BR, Hickman-Brenner F, et al. Biochemical identification of new species and biogroups of Enterobacteriaceae isolated from clinical specimens. J Clin Microbiol. 1985;21:46-76.
47. Sanders W, Sanders CC. Enterobacter spp.: pathogens poised to flourish at the turn of the century. Clin Microbiol Rev. 1997;10:220-241.
48. Munez E, Ramos A, de Espejo Alvarez T, et al. Aetiology of surgical infections in patients undergoing craniotomy. Neurocirugia (Astur). 2012;23:54-9.
49. Wang H, Shi H, Zhou W, et al. Common pathogens and clinical characteristics of neonatal pneumonia. Zhongguo Dang Dai Er Ke Za Zhi. 2012;14:898-902.
50. Edlin RS, Shapiro DJ, Hersh AL, et al. Antibiotic resistance patterns of outpatient pediatric urinary tract infections. J Urol. 2013;190:222-227.
51. da Silva CLP, Miranda LEV, Moreira BM, et al. Enterobacter hormaechei bloodstream infection at three neonatal intensive care units in Brazil. Pediatr Infect Dis J. 2002;21:175-177.
52. Grimont PA, Grimont F. Enterobacter. Bergey's Manual of Systematics of Archaea and Bacteria, 2015:1-17.
53. Davin-Regli A, Bolla J-M, James CE, et al. Membrane permeability and regulation of drug "influx and efflux" in enterobacterial pathogens. Curr Drug Targets. 2008;9:750-759.
54. Jacoby GA. AmpC β-lactamases. Clin Microbiol Rev. 2009;22:161-182.
55. Diene SM, Merhej V, Henry M, et al. The rhizome of the multidrug-resistant Enterobacter aerogenes genome reveals how new "killer bugs" are created because of a sympatric lifestyle. Mol Biol Evol. 2013;30:369-383.
56. Hunter CJ, Petrosyan M, Ford HR, et al. Enterobacter sakazakii: an emerging pathogen in infants and neonates. Surg Infect (Larchmt). 2008;9:533-539.
57. Muytjens HL, Zanen H, Sonderkamp HJ,et al. Analysis of eight cases of neonatal meningitis and sepsis due to Enterobacter sakazakii. J Clin Microbiol. 1983;18:115-120.
58. Chenu J, Cox J. Cronobacter ('Enterobacter sakazakii'): current status and future prospects. Lett Appl Microbiol. 2009;49:153-159.
59. Cruz A, Xicohtencatl-Cortes J, González-Pedrajo B, et al. Virulence traits in Cronobacter species isolated from different sources. Can J Microbiol. 2011;57:735-744
60. Parra-Flores J, Aguirre J, Juneja V, et al. Virulence and antibiotic resistance profiles of Cronobacter sakazakii and Enterobacter spp. involved in the diarrheic hemorrhagic outbreak in Mexico. Front Microbiol. 2018;9:2206.
61. Yan Q, Condell O, Power K,et al. Cronobacter species (formerly known as Enterobacter sakazakii) in powdered infant formula: a review of our current understanding of the biology of this bacterium. J Appl Microbiol. 2012;113:1-15.
62. Drudy D, Mullane N, Quinn T, et al. Enterobacter sakazakii: an emerging pathogen in powdered infant formula. Clin Infect Dis. 2006;42:996-1002.
63. Bar-Oz B, Preminger A, Peleg O,et al. Enterobacter sakazakii infection in the newborn. Acta Paediatr. 2001;90:356-358.
64. Mullane N, Healy B, Walsh C, et al. Enterobacter Sakazakii: an Emerging Microbe With Implications for Infant Health. Minerva Pediatr. 2007; 59:137-148.
65. Healy B, Cooney S, O'Brien S, et al. Cronobacter (Enterobacter sakazakii): an opportunistic foodborne pathogen. Foodborne Pathog Dis. 2010;7:339-350.
66. Jaradat ZW, Ababneh QO, Saadoun IM, et al. Isolation of Cronobacter spp.(formerly Enterobacter sakazakii) from infant food, herbs and environmental samples and the subsequent identification and confirmation of the isolates using biochemical, chromogenic assays, PCR and 16S rRNA sequencing. BMC Microbiol. 2009;9:225.
67. Organization WH. Safe preparation, storage and handling of powdered infant formula: guidelines, 2007.
68. Bowen AB, Braden CR. Invasive Enterobacter sakazakii disease in infants. Emerg Infect Dis. 2006;12:1185-1189.
69. Jvo Siegrist. Chronobacter spp. Classic and New Detection Methods. Microbiology Focus Edition 1.2.
70. Cetinkaya E, Joseph S, Ayhan K, et al. Comparison of methods for the microbiological identification and profiling of Cronobacter species from ingredients used in the preparation of infant formula. Mol Cell Probes. 2013;27:60-64.
71. Joseph S, Hariri S, Forsythe SJ. Lack of continuity between Cronobacter biotypes and species as determined using multilocus sequence typing. Mol Cell Probes. 2013;27:137-139.
72. Erickson MC, Kornacki JL. Enterobacter sakazakii: an emerging food pathogen. Surg Infect (Larchmt). 2008; 9: 533–539.
73. Lehner A, Stephan R. Microbiological, epidemiological, and food safety aspects of Enterobacter sakazakii. J Food Prot. 2004;67:2850-2857.
74. Townsend SM, Hurrell E, Caubilla-Barron J, Loc-Carrillo C, Forsythe SJ. Characterization of an extended-spectrum beta-lactamase Enterobacter hormaechei nosocomial outbreak, and other Enterobacter hormaechei misidentified as Cronobacter (Enterobacter) sakazakii. Microbiology. 2008;154:3659-3667.
75. Iversen C, Lehner A, Mullane N, et al. The taxonomy of Enterobacter sakazakii: proposal of a new genus Cronobacter gen. nov. and descriptions of Cronobacter sakazakii comb. nov. Cronobacter sakazakii subsp. sakazakii, comb. nov., Cronobacter sakazakii subsp. malonaticus subsp. nov., Cronobacter turicensis sp. nov., Cronobacter muytjensii sp. nov., Cronobacter dublinensis sp. nov. and Cronobacter genomospecies 1. BMC Evol Biol. 2007 17;7:64.
76. Stock I, Wiedemann B. Natural antibiotic susceptibility of Enterobacter amnigenus, Enterobacter cancerogenus, Enterobacter gergoviae and Enterobacter sakazakii strains. Clin Microbiol Infect. 2002;8:564-578.
77. Friedemann M. Epidemiology of invasive neonatal Cronobacter (Enterobacter sakazakii) infections. Eur J Clin Microbiol Infect Dis. 2009;28:1297-1304.
78. Brenner DJ, Staley JT, Krieg NR. Classification of Procaryotic Organisms and the Concept of Bacterial Speciation. Bergey's Manual® of Systematic Bacteriology pp 27-32.
79. Iimura K, Hosono A. Biochemical characteristics of Enterobacter agglomerans and related strains found in buckwheat seeds. Int J Food

Microbiol. 1996;30:243-253.
80. Cruz AT, Cazacu AC, Allen CH. Pantoea agglomerans, a plant pathogen causing human disease. J Clin Microbiol. 2007;45:1989-1992.
81. Büyükcam A, Tuncer Ö, Gür D, et al. Clinical and microbiological characteristics of Pantoea agglomerans infection in children. J Infect Public Health. 2018;11:304-309.
82. Aly NYA, Salmeen HN, Lila RAA, et al. Pantoea agglomerans bloodstream infection in preterm neonates. Med Princ Pract. 2008;17:500-503.
83. Siwakoti S, Sah R, Rajbhandari RS, et al. Pantoea agglomerans Infections in Children: Report of Two Cases. Case Rep Pediatr. 2018;2018:4158734.
84. Mardaneh J, Dallal MMS. Isolation, identification and antimicrobial susceptibility of Pantoea (Enterobacter) agglomerans isolated from consumed powdered infant formula milk (PIF) in NICU ward: First report from Iran. Iranian Journal of Microbiology 2013 Vol.5 No.3 pp.263-267.
85. Rezzonico F, Smits TH, Montesinos E, et al. Genotypic comparison of Pantoea agglomerans plant and clinical strains. BMC Microbiol. 2009;9:204.
86. DeLucca A, Brogden K, Engen R. Enterobacter agglomerans lipopolysaccharide-induced changes in pulmonary surfactant as a factor in the pathogenesis of byssinosis. J Clin Microbiol. 1988;26:778-780.
87. Martinez J, Toval M, Colomo L. 3 new cases of Enterobacter taylorae infection. Enferm Infecc Microbiol Clin. 1994;12:289-292.
88. Garazzino S, Aprato A, Maiello A, et al. Osteomyelitis caused by Enterobacter cancerogenus infection following a traumatic injury: case report and review of the literature. J Clin Microbiol. 2005;43:1459-1461.
89. Doijad S, Imirzalioglu C, Yao Y, Pati NB, Falgenhauer L, Hain T, et al. Enterobacter bugandensis sp. nov., isolated from neonatal blood. Int J Syst Evol Microbiol. 2016;66:968-974.
90. Weinstein RA. Nosocomial infection update. Emerg Infect Dis. 1998; 4: 416–420.
91. Stoesser N, Sheppard A, Shakya M, Sthapit B, Thorson S, Giess A, et al. Dynamics of MDR Enterobacter cloacae outbreaks in a neonatal unit in Nepal: insights using wider sampling frames and next-generation sequencing. J Antimicrob Chemother. 2015;70:1008-1015.