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Antimicrobial susceptibility of Citrobacter Koseri isolated on clinical samples of hospitalized patients

Abstract

The genus *Citrobacter* includes 13 species and is often found in water, food, land and certain animals. It is part of the normal flora of a small proportion of healthy humans; however, the rates of colonization increase in patients in long-term care institutions and hospitals. *Citrobacter koseri* is an aerobic Gram-negative bacillus, ubiquitous in nature, opportunistic, that is frequently found in genitourinary and gastrointestinal tracts of animals and humans as a saprophyte flora. *Citrobacter koseri* is one of the most important pathogens, eventually causing epidemics difficult to control. Therefore, the objective of this article is to carry out a microbiological characterization of this pathogen. A transversal descriptive study was conducted in the Surgical Clinical Hospital Lucía Íñiguez Landín of Holguín, which covered the period from January to December 2019. The study universe consisted of all enterobacteriaceae isolated in clinical samples of adult patients admitted during this period and the sample was formed by 219 strains of *Citrobacter koseri*. Out of the total number of cases, 70 belonged to the intensive care service for a 32.0%. Out of the total number of samples, 139 were isolated in pus samples for a 63.5%. The strains were more resistant to ciprofloxacin, cefuroxime, ampicillin, aztreonam, ceftazidime and to gentamicin. *Citrobacter koseri* is able to develop multidrug resistance to known antibiotics, acts as opportunistic and can colonize people in community, consequently its dissemination should be controlled.

Keywords: *Citrobacter koseri*, nosocomial infections, antimicrobial susceptibility, resistance mechanisms

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Introduction

The genus *Citrobacter* includes 13 species, of which the most frequently isolated are: *C. freundii, C. koseri* and *C. amalonaticus*, so named due to their ability to use citrate as their only carbon source. They differ because of their ability to convert tryptophan into indole, to ferment lactose and use malonate.[1] The gene Blacko (encodes the chromosomal class A B-lactamase CKO) is only present in *C. koseri*; therefore, it represents an interesting means to differentiate it from other species of *Citrobacter*. *C. freundii* and *C. koseri* cause most infections by *Citrobacter*, which have epidemiology and clinical manifestations similar to those of enterobacter infections.[1] The genus *Citrobacter* is often found in water, food, land and certain animals. It is part of the normal flora of a small proportion of healthy human beings; however, colonization rates increase in patients in long-term care institutions and hospitals, provoking 1 to 2% of nosocomial infections. Affected hosts usually have immunosuppression or concomitant diseases. According to a large observational study, *Citrobacter spp*. account for 0.8% of all Gram-negative infections in a hospital setting, with a mortality rate in hospitalized patients that ranged from 6.8% to 56%. *Citrobacter* causes extraintestinal infections similar to those caused by other Gram-negative bacilli. Infections caused by multidrug-resistant *Citrobacter* strains are associated with a higher rate of in-hospital mortality compared to those caused by susceptible strains.[1-3] *C. koseri* is an aerobic Gram-negative bacillus, ubiquitous in nature and opportunistic, which is frequently found in genitourinary and gastrointestinal tracts of animals and humans as a saprophyte flora. These microorganisms can produce serious infections, especially in immunocompromised hosts; with greater preference for the central nervous system; they are associated with meningitis, cerebral abscesses, ventriculitis, panophthalmitis and sepsis. Most cases are sporadic, without a clear source of infection. *C. koseri* is one of the most important pathogens, eventually causing epidemics difficult to control. It has been described that in other anatomical sites it destroys microvilli, causing characteristic adhesion and elimination injuries.[4,5] *C. koseri* has a susceptibility pattern to the antibiotics similar to that of Klebsiella (resistant to ampicillin and ticarcillin), is sensitive to ciprofloxacin, carbapenems, third-generation cephalosporins, piperaciline-thazobactam, aminoglycosides and trimethoprim-sulfamethoxazole.[6,7]

Nosocomial infections are an important cause of high morbidity and mortality in the health care system. Those caused by resistant microorganisms generate nosocomial outbreaks difficult to eradicate. This increase in infection is due to several reasons: the disease of the patient that causes a state of immunosuppression, the use of broad-spectrum antibiotics as well as the use of steroids, the instruments derived from new technologies, the long-term use of parenteral nutrition, among others. In the present study, *C. koseri* is the main pathogen responsible for nosocomial infections; it is alarming because it is not an isolated bacterium in large quantities of clinical samples and its resistance pattern shows that eventually we may not have resources to confront it. Therefore, the objective of this article is to carry out a microbiological characterization of this pathogen.

Materials and methods

A tranversal descriptive study was carried out at the Surgical Clinical Hospital Lucía Íñiguez Landín of Holguín, which covered the period from January to December 2019. The study universe consisted of all enterobacteriaceae isolated on clinical samples of adult patients admitted during this period and the sample was formed by 219 strains of *Citrobacter koseri*.

Inclusion criteria: All patients included in the study were selected by the positivity of their samples.

Exclusion criteria: contaminated or non-viable samples for study.

The variables analyzed in each isolation were: service in which it was isolated, type of sample, and the level of resistance, all polychotomus nominal qualitative.

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The methodology used to identify the *Citrobacter koseri* was the following:

Biological samples: purulent, hemocultures, respiratory secretions, otic exudates, biliary liquid, catheter, sputum, ascitic fluid, cerebrospinal fluid, peritoneal fluid, abdominal fluid, urine culture.

Materials and culture media: Graduated culture incubator of 35 to 37ºC inoculation loops and needles. Gram stain sets. Plates for serology. Lab microscope slides. Blood Agar Plates 5%, Mac Conkey Agar, SS agar, XLD agar, nutrient agar, Müeller-Hinton agar for antibiogram, that will be used based on the type of sample, whether it is intestinal or extraintestinal infections. Oxidase reagent.

Tests for biochemical identification: Klighler, Lia, Mio, Simmons citrate, Christensen urea. Phenylalanine, sodium malonate, Voges Proskauer, sorbitol. Polivalent antisera of Citrobacter. Container with a lid with 0.5% sodium hypochlorite solution, for the tank of the discard material of the serological tests.

Direct examination: Useful samples to be researched are extended and stained with Gram, observing gram-negative, characteristic bacillus.

Identification: Biochemical tests are used for identification of gender and species, according to the attached tables and flowcharts.

Antibiogram: The susceptibility proof is performed through the dissemination method with the Kirby and Bauer technique. The standards to determine the susceptibility to the different antibiotics, were taken from the recommendations of the Clinical and Laboratory Standards Institute, using the standardized method of disk broadcasting (known as Bauer-Kirby), using non-supplementing Mueller-Hinton agar with NaCl.[8]

The data was obtained from both the records and antibiograms of the microbiology laboratory and the medical records of patients diagnosed with *Citrobacter koseri* through a worksheet that gathered the origin of the samples and the service of origin, as well as the laboratory results and the antibiotic sensitivity for 29 antibiotics, Amikacin, Ampicillin, Ampicillin/Sulbactam, Cefazoline, Cefepime, Ceftazidime, Cephtriaxone, Tobracycin, Piperacylin, Amoxicillin, Piperacylin/Tazobactam, Cefotaxime, Cefuroxime, Aztreonam, Azithromycin, Meropenem, Gentamycin, Kanamicin Ciprofloxacin, Norfloxacin, AC. Nalidixic, Trimethoprim/Sulfametoxazole, Chloramphenicol, Nitrofurantoin, Phosphomycin, Streptomycin, Tetracycline, Doxycycline, Augmentín; they presented 3 values: resistant (R), intermediate (I) and sensitive (s).

Based on this spreadsheet, a database was developed, using Microsoft Office Access 2010, which allowed the statistical analysis of the variables. The information was summarized in tables using the Microsoft Office Excel 2010 program for a better understanding and analysis. With the data obtained, simple frequency distribution tables were elaborated and the results were expressed in whole numbers and percentages.

Results

Table 1 shows the distribution according to hospitalization services. Out of the total number of cases, 70 belonged to the intensive care service for a 32.0%.

Table 1 Distribution according to hospitalization services

Services	No	%
ICU	70	32
Kidney Transplant	6	2.7
Angiology	25	11.4
Hemodialysis	1	0.5
Intermediate Therapy	17	7.8
Urology	16	7.3
Ophthalmology	1	0.5
Dermatology	4	1.8
Neurosurgery	11	5
Neurology	3	1.4
Surgery	29	13.2
Orthopedics	24	11
ORL	2	0.9
Hematology	4	1.8
Internal Medicine	6	2.7
Total	219	100

ICU, intensive care unit; ORL, otorhinolaryngology

Source: Microbiology records.

Table 2 shows the distribution according to the type of sample where the strains were isolated. Out of the total number of samples, 139 were isolated in pus samples for a 63.5%.

Table 2 Distribution according to type of sample

Sample	No	%
Purulent	139	63.5
Blood culture	19	8.7
Respiratory secretions	23	10.5
Otic Exudate	2	0.9
Bile Fluid	2	0.9
Catheter	12	5.5
Sputum	1	0.4
Ascitic fluid	1	0.4
Cerebrospinal fluid	3	1.4
Peritoneal fluid	5	2.3
Abdominal fluid	2	0.9
Urine culture	10	4.6
Total	219	100

Source: Microbiology records

The tables show the susceptibility profile for the different antibiotics. The strains showed greater resistance to ciprofloxacin in table 3, in table 4 to cefuroxime, ampicillin, aztreonam and ceftazidime, and in table 5 gentamicin was the predominant antibiotic in terms of resistance.

Discussion

Documentation of medical institutions and health care services regarding nosocomial infections, registered around the world, report that they are an important cause of morbidity and mortality. The high frequency of these infections ascertains the poor quality in the provision of health care services, which also causes high avoidable costs.

Table 3 *Citrobacter koseri* susceptibility profile

Antibiotics	Symbol	S	I	R
Azithromycin	AZM	1	0	23
Ciprofloxacin	CIP	39	2	98
Norfloxacin	NOR	5	0	7
Ac. Nalidixic	NA	11	1	63
Trimethoprim/ Sulfametoxazole	SXT	9	0	42
Chloramphenicol	C	25	0	50
Nitrofurantoin	F	38	2	43
phosphomycin	FOS	60	7	40
Tetracycline	TE	0	0	1
Doxycycline	DXT	22	1	54

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Table 4 Betalactam susceptibility profile

Antibiotics	Symbol	S	I	R
Ampicillin	AMP	1	0	114
Piperacilin	PIP	17	1	63
Amoxicillin	AML	0	0	91
ampicillin / sulbactam	AMS	14	0	91
Piperacilin / Tazobactam	TZP	55	4	78
Augmentín	AUG	3	1	20
Cefazoline	CFZ	8	0	57
Cefepime	FEP	45	2	88
Cefotaxym	CTX	20	0	78
Ceftriaxone	CRO	19	2	84
Ceftazidima	CAZ	23	2	102
Cefuroxym	CXM	22	4	116
Meropenem	MRP	80	3	51
Aztreonam	ATM	50	5	109

Source: Microbiology records

Table 5 Aminoglycoside susceptibility profile

Antibiotics	Symbol	S	I	R
Gentamycin	GEN	51	2	120
Amikacin	AMK	56	4	35
Kanamycin	K	17	2	64
Tobramycin	TOB	19	1	36
Streptomycin	S	6	1	5

Source: Microbiology records

Several factors increase the frequency of infections associated with healthcare. Hospitalized patients often get immunocompromised; they undergo a vast number of medical examinations and treatments, most of them invasive. Health care procedures and the setting of the hospital allow the transmission of microorganisms between the hosts.[9] In this study, the service with more strains was ICU, the patients are mostly inmunocompromised, they are subjected to invasive procedures, they are constantly manipulated by medical and nursing staff, and most patients are intubated. These are risk factors that influence the cause of infection.

Despite *Citrobacter* species are considered an unusual nosocomial pathogen, neonates and inmunocompromised patients are a frequent target of infections caused by these microorganisms. These conditions include sepsis, urinary tract infections, respiratory and intra-abdominal infections, and central nervous system Infections.[1,11,12]

C. koseri is a pathogen mostly isolated in urinary samples;[12,13] however, this was not the case of the present study, since it was isolated in more than 50% of pus samples, which classifies it as a pathogen responsible for nosocomial infections.

Antimicrobials are the fundamental basis in the treatment of an infection, which is one of the most frequent problems in the health care systems, and the cause of greatest morbidity and mortality in any medical specialty.[14-16]

The selective pressure of antimicrobials, produced mostly by bacteria of environmental origin, led to a logical coevolution: the producing microorganisms, or those who share their ecological niche, developed resistance mechanisms to those same compounds as part of their own subsistence. Thus, the presence of antibiotics in a given environment not only selects changes in the characteristic genes of species that seek to survive their action, but also favors the lateral dissemination of these mechanisms from its original hosts to other bacterial species through mobile genetic elements such as transposons and plasmids. In the hospital setting, there is a great antibiotic selective pressure due to the use of broad-spectrum antimicrobials; therefore, it is not surprising the emergence of multidrug resistant pathogens. The use of antibiotics makes it even more difficult to control their rational use and generates a new source of selection of resistant bacteria that can disseminate later through food or the environment, as well as colonize the digestive tract of human beings. As a result, we are heading towards a period similar to that of when there were no antibiotics, with bacterial infections for which there is no treatment available, or in which alternatives are far from ideal (for example, old antibiotics in disuse due to their high toxicity, such as the polymyxins). Taking into account the scarce number of new developing antibiotics, the detection and monitoring of multidrug resistant bacteria becomes fundamental in order to define actions that improve the use of available antimicrobials and prevent the dissemination of resistant pathogens.[17]

Antimicrobial resistance is a global problem that has caused current therapeutics not to solve bacterial infections. Within the most important and high-impact current resistance mechanisms is the resistance to carbapenems in enterobacteriaceae (CRE), emerged as in most cases, as a consequence of the indiscriminate use of antibiotics in medicine. Among the enzymes capable of hydrolyzing the carbapenems and other β-lactam, can be underlined: Class A of Ambler: KPC-2 to 18; Class B: metallotamases (VIM 1 to 41 and IMP 1 to 48, and NDM-1 to NDM-12 and Class C: (detected in *Acinetobacter spp.)* OxA-23, OXA-25 to 27, OXA -40, OXA-51, OXA- 55, OXA-58 and OXA-143 (4).[18]

There is a broad family of bactericidal antibiotics, and betalactamics are one of the most numerous groups and of greater clinical use; they include penicillins, cephalosporins, monobactams and carbapenems. Although the resistance to betalactamics is defined by different mechanisms (production of enzymes, alterations of the permeability, alteration of the target and, presumably, expression of active expulsion pumps), the main mechanism of resistance to betalactamics in enterobacteriaceae is enzymatic, due to the production of betalactamases.

C. koseri, presents low-level resistance to aminonicilines (ampicillin) and carboxypenicycilines (ticarcilin) and decreased or intermediate sensitivity to ureidopenicillins (piperacilin), remaining sensitive to cephalosporins, monobactam (aztreonam), carbapenemic (imipenem) and associations with inhibitors of Betalactamase (Amoxicillin-AC. clavulanic) The hyperproduction of chromosomal beta-lactamase of Class A: This phenotype can be found in *C. Koseri*.[7,13] In this research *C. koseri* presented high-level resistance to ampicillin, ceftazidime, cefuroxime and aztreonam, as well as moderate resistance to all antibiotics that were used in the study.

The behavior of an aminoglucoside before enterobacteria depends, at least, of 5 factors: a) passive diffusion through the outer membrane; b) active transportation through the inner membrane; c) the affinity of aminoglycoside for its target (a ribosomal protein); d) The methylation of unit 16 s of ribosomal RNA, and e) the presence of inactivating enzymes. However, the most important mechanism of resistance to aminoglycosides remains enzymatic inactivation. Three types of enzymes have been described: acetyltransferases (AAC) that produce the acetylation of an amino group of the antibiotic, phosphotransferases (APH) that phosphorylate a hydroxyl group and, finally, nucleotidyltransferases (ANT) that also adequate a hydroxyl group. Each enzyme recognizes a number of antibiotics aminoglycosides, which results in a phenotype of particular resistance. Most species of enterobacteria are naturally sensitive to aminoglycosides, AAC (20) confers resistance to gentamicin, tobramycin, netilmicin and neomycin, while the AAC enzyme (60) confers only a slight resistance to tobramicin, observing in the antibiogram by disk-diffusion zones of inhibition more reduced than for the rest of enterobacteriaceae, halos

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corresponding to CIM for this antibiotic. Mutations in this gene cause a hyperproduction of the enzyme that confers a high-level resistance to tobramicin, kanamycin and netilmicin and moderate to amikacin.[7] For instance, *Oxalis corniculata* extract has intense antimicrobial activity against *C. koseri*.[19,20]

In this study *C. koseri* showed a high resistance to gentamicin, which is alarming since, as mentioned above, enterobacteriaceae are sensitive to aminoglycosides, besides the fact that this therapeutic option would no longer be available.

Conclusion

Citrobacter koseri is a bacterium that has always been frequently isolated in urinary tract infections, although is currently causing outbreaks of nosocomial infections, mainly, in intensive care units. Its isolation in purulent samples demonstrates skin colonization either by primary cause infections or transmitted by health care staff as it seems to be the case in this research. These many strains of *Citrobacter koseri* had been isolated in this institution in the period of a year, since *Citrobacter freundii* is the most studied species in clinical samples. It is now clear that *Citrobacter koseri* is able to develop multi-resistance to known antibiotics, acts as opportunistic and can colonize people in community, thus its dissemination should be controlled. The antibiotic that is being used to treat this infection is the colistin due to its efficiency in multi-resistance by *Citrobacter freundii;* this is the alternative considered so far. Due to its significance, this research is part of a developing institutional project to continue the study of other microbiological characteristics of this bacterium.

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None.

Conflicts of interest

There are no conflicts of interest between the authors.

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