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The cephalosporin-resistant bacterial strains from urinary tract

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Abstract

Urinary tract infections are considered of great relevance among infections of bacterial origin as one of the third most important bacterial infections worldwide. These infections are caused by bacteria belonging to the Enterobacteriaceae family, such as *Escherichia coli*, which is the most frequently isolated uropathogen. The objective was to determine the level of resistance to cefotaxime and ceftazidime in bacterial strains from urinary tract infections. Material and methods. The study strains were isolated from patients who presented urinary tract infections, the genus and species were ratified by conventional microbiological methods. The antimicrobial sensitivity test was performed using the Kirby Bauer method and minimum inhibitory concentration, testing β-lactams that belong to the family of third-generation cephalosporins (cefotaxime and ceftazidime). The presence of extended-spectrum β-lactamases was searched for by the combined disc method. Forty-three bacterial strains from a collection of strains from the Microbiology Laboratory of the Faculty of Chemical Sciences, BUAP, were analyzed. All the strains studied showed resistance to the antimicrobials tested and production of the extended-spectrum β-lactamase enzyme. This study showed that *E. coli* continues to be the most frequent uropathogen responsible for urinary tract infections. It also showed the participation of resistance mechanisms, such as the presence of extended-spectrum β-lactamases, which confer high resistance values to third generation cephalosporins, reflecting the need to implement measures for the use of antimicrobials in our community.

Keywords: Cefotaxime; Ceftazidime; *Escherichia coli*; Extended-spectrum β-lactamases; Resistance mechanisms; UTI

1. Introduction

Urinary tract infections (UTIs) are the most common community-acquired infection. It is estimated that 40% of women and 12% of men will have at least one episode of UTI in their adult life [1]. It has been reported in the US that this disease has accounted for approximately eight million emergency room or clinic visits, and 100,000 hospital admissions [2]. Women have a higher probability of suffering from a UTI during their lives, including pregnancy. On the other hand, in men, the incidence is notably from the third age. The frequency of these infections in people with diabetes is higher than in the general population [3]. Within a year of an acute urinary infection, 27% to 46% of women will have another UTI, in addition, age is an important factor because, in older women, estrogen diminishes and pH increases, thus promoting colonization of the vagina and perineum by bacteria [4]. In women, over 65 years of age it is about 20%, compared to 11% in the general population. In addition, between 50% and 60% of adult women will have at least one UTI in their lifetime, and about 10% of postmenopausal women have had a UTI in the past year [5]. Populations with the greatest risks of contracting UTI are newborns, girls of age preschool, sexually active women, and older people of both genders. In Mexico, UTIs are a public health problem since every year it has recorded approximately four million cases [6].

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The UTIs do not always present with clinical manifestations since they can be asymptomatic, but fever and pain can sometimes occur in one or both lumbar fossae, considering pyelonephritis, dysuria, and frequency, which would be typical of cystitis. UTIs present many clinical manifestations that will depend on the degree of the pathogenesis of the causal agent, the geographical area, the patient's condition, and treatment [7].

Urinary tract infections are caused by bacterial invasion of the urothelium of the bladder, from bacteria migrating from the rectum as well as colonized bacteria from the perineum and vagina [4]. Most of the causative agents of UTIs are bacteria of enteric origin, where 93% are gram-negative bacilli, 6% gram-positive cocci, and 1% yeasts, viruses, protozoa, or parasites. The most frequently isolated microorganism is *Escherichia coli*, which causes 79% to 95% of community-acquired UTIs [8], other members of the Enterobacteriaceae family include *Klebsiella* sp, *Proteus* sp, and *Enterobacter* sp, as well as *Pseudomonas* sp has also been found, particularly when the patient has recurrent, nosocomial, or treatment-complicated diseases or if the patient has undergone some type of surgical instrumentation [9]. *Enterococcus faecalis* and group B S*treptococcus* are known to cause complicated and uncomplicated UTIs. Another agent is *S. saprophyticus*, which can cause infections in young women who have an active sexual life, leading to another complication, such as cystitis. Coagulase-positive *Staphylococcus* can invade the kidney by hematogenous dissemination and cause renal abscesses. Finally, a pathogen found in *Corynebacterium urealyticum* is frequently isolated in patients with chronic idiopathic prostatitis, in addition, *Candida* may become the second microorganism responsible for nosocomial UTIs [10,11]. Urethral catheterization accounts for 80% of nosocomial UTIs; 5% to 10% are related to genitourinary manipulations and sexual intercourse results in an increased risk, as does the use of a diaphragm or spermicide [12,13]. The development of antibiotic resistance is a natural and inevitable process. However, the last decades have witnessed how the use of antibiotics has resulted in antibiotic multi-resistant strains in hospitals and community environments. Antibiotic research and the development of new antibiotic has stopped at a time when treatment failure is constantly manifesting itself by increasing the economic and human life cost [14].

Antimicrobial resistance is a problem of the future that we are currently witnessing; failure to stop it will lead to a situation that can be dramatic. Awareness should be raised not only in the medical profession but also in the general population, educating patients about the appropriate use of antimicrobials and the potential harm of antimicrobial treatment, to curb the future crisis that is being managed. A future without antibiotics is a future of super bacteria and supportive treatments with high mortalities [15].

Urinary tract infections (UTIs) are considered one of the most frequent; these infections have now been treated with various antibiotics including β-lactams. However, these infections have been prolonged, and treatments are often ineffective, resulting in represents a serious concern for drug treatment. Infections by bacteria producing extended-spectrum β-lactamases (ESBL) represent a serious challenge for modern healthcare systems and are associated with higher mortality rates and high costs related to health care. The problem has increased notably, mainly due to excessive use or misuse of antibiotics, along with the development of a few new drugs by industry pharmaceutical, in addition to the production of β-lactamase enzymes, as they affect the action of β-lactam antimicrobials and other families of antimicrobials [16]. Besides this, in Mexico exist a small number of networks specialized in specific pathologies and indicators in other to know the real impact of antimicrobial resistance. The use of new diagnostic methods will be of vital importance in their management, as they can offer therapies directed to the pathogen in a timelier manner and avoid the antimicrobial resistance that is one of the great conflicts today [5]. The World Health Organization (WHO) proposes an urgent action plan based on awareness about the problem, reinforcement of knowledge, and reduction of incidence of infections through preventive measures thus preventing that bacterial resistance will cause 10 million deaths by 2050. As part of the Global Action Plan on Antimicrobial Resistance, it also proposed networks of specialized laboratories to preserve strains and optimize the use of antimicrobials such as environmental sanitation, hand washing, and the optimal use of antimicrobials, both in humans as well as animals [17]. The objective was to determine the level of resistance to cefotaxime and ceftazidime in bacterial strains from urinary tract infections.

2. Material and methods

2.1. Bacterial strains and culture conditions

Forty-three clinical isolates were obtained from a collection of strains from the Laboratory of Microbiology of the Faculty of Chemical Sciences, BUAP, which were isolated from patients who presented urinary tract infection, during the period August-December 2018. The isolates were identified by conventional microbiology tests. As reference strains, *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used.

2.2. Antimicrobial susceptibility testing and determination of the minimum inhibitory concentration (MIC)

The antimicrobial susceptibility testing was done with third-generation cephalosporins using test discs of cefotaxime, CTX, and ceftazidime, CAZ (BD BBL™ Sensi-Disc™) according to the disc-diffusion method described by Kirby-Bauer method [18]. The bacterial inoculum was prepared by suspending the microorganism in a tube with sterile isotonic saline solution until a turbidity corresponding to the 0.5 standards of the MacFarland nephelometer equivalent to 1.5 X 10^8 CFU/mL. The Mueller-Hinton agar plates were inoculated with a swab dipped into the bacterial suspension, taking care to remove the excess inoculum. The inoculum was spread over the plate in 3 different directions in order to completely cover the entire surface of the agar to achieve uniform growth and was allowed to dry for 10 minutes at room temperature, the impregnated filter paper discs with cefotaxime (30 µg) and ceftazidime (30 µg) were placed. The plates were incubated from 16 to 18 hours at 35°C. The reading of the diameter of the zone of inhibition obtained was interpreted as resistant, sensitive, or intermediate according to the recommendations of the CLSI (Clinical and Laboratory Standards Institute) [19] considering the zone diameter breakpoint ≤22 mm as resistant-cefotaxime and ≤17 mm as resistant-ceftazidime.

The MICs were determined in the strains by the agar dilution method. Plates were prepared on the day of use with 1:2 dilutions from a concentration of 2 µg/mL to 1024 µg/mL of each of the antimicrobial agents to be tested. A control plate without antibiotics was used. The plates were inoculated with 1-2 µL of a 1:10 suspension prepared in sterile distilled water from a bacterial suspension corresponding to the 0.5 MacFarland nephelometer standard, on the surface of the agar. The inoculum was allowed to absorb into the agar prior to incubation. The MIC was defined as the lowest concentration of antibiotic at which there was no visible growth, ensuring that all bacteria had grown on the antibiotic-free control plate.

This plate dilution technique based on the breakpoints recommended by the Clinical and Laboratory Standard Institute (CLSI), considering the breakpoint ≥4 µg/mL as resistant-cefotaxime and the breakpoint ≥16 µg/mL as resistant-ceftazidime [19].

2.3. Detection of ESBL-producing strains by the combined disc method

In this study, for the detection of ESBL-producing strains, the combined disc method was used. Muller Hilton plates were inoculated in the same way as in the previous section, the discs previously impregnated with cefotaxime 30 µg, ceftazidime 30 µg, cefotaxime-clavulanic acid (CTX/CLA 30/10 µg), and ceftazidime-clavulanic acid (CAZ/CLA 30/10 µg) were placed on the agar. Placed the antibiotic disc and the antibiotic plus inhibitor disc at a distance of 2.5 cm. The plates were incubated from 18 to 20 hours at 35-37 ^{0}C. In all cases, the growth inhibition halo was measured (mm). Clavulanic acid is an enzyme inhibitor ß-lactamases, according to the CLSI criteria they were producing bacteria of ESBL those with a diameter of inhibition greater than or equal to 5 mm, with respect to the size of the halo with the disc with cephalosporins without clavulanic acid, it was considered positive. In all sensitivity studies reference antimicrobial strains, *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were routinely included.

3. Results

The 43 bacterial isolates were recovered from patients with urinary tract infections as mentioned in the Methodology. The results obtained are shown in Table 1. As you can see, 95% of the isolates were identified as *E. coli* (41/43) and 5% as *K. pneumoniae* (2/43). The identified bacterial species coincided with the reference strains used.

The antimicrobial susceptibility was determined. For this, the minimum inhibitory concentration was measured. The results obtained are also shown in Table 1, where it is appreciated that all bacterial isolates showed resistance to CTX and CAZ. Of 43 bacterial isolates that were exposed to different concentrations of CTX, 5 showed resistance at a MIC value of 256 µg/mL. At concentrations lower than 256 µg/mL, all bacterial isolates showed growth. Also, 13 bacterial isolates showed resistance at a MIC value of 512 µg/mL and the rest of the bacterial isolates (25) showed the highest resistance to CTX, reaching a MIC value of 1024 µg/mL.

Of 43 bacterial isolates that were exposed to different concentrations of CAZ, 2 showed resistance at a MIC value of 8 µg/mL. At concentrations lower than 8 µg/mL, all bacterial isolates showed growth. 95% (41/43) of bacterial isolates showed resistance with a MIC value of ≥16 µg/mL, including 39 strains of *E. coli* and 2 strains of *K. pneumoniae*. In addition, four strains showed high resistance to CAZ, they reach a MIC value of 1024 µg/mL (Table 1).

On the other hand, the ESBL-producing strains were detected using the combined disc method. For that, discs were impregnated with cephalosporins and clavulanic acid. In this study, 43 resistant strains to CTX and CAZ showed ESBL

production (the results corresponded with the controls used). The strains tested presented an inhibition halo ≥ 5mm of CTX-CLA and CAZ-CLA with respect to CTX and CAZ alone (Table 2 and Figure 1).

Table 1 The minimum inhibitory concentrations of bacterial isolates

Strain isolate	MIC µg/mL	MIC µg/mL	Strain isolate	MIC µg/mL	MIC µg/mL
	CTX	CAZ		CTX	CAZ
EC1	512	64	EC22	1024	32
EC2	256	8	EC23	1024	8
EC3	1024	64	EC24	1024	64
EC4	256	64	EC25	1024	16
KP1	1024	16	EC26	512	64
EC5	512	256	EC27	1024	128
EC6	1024	32	EC28	1024	128
EC7	1024	1024	KP2	256	64
EC8	1024	256	EC29	1024	64
EC9	512	16	EC30	512	128
EC10	512	32	EC31	1024	64
EC11	1024	1024	EC32	512	32
EC12	1024	64	EC33	1024	1024
EC13	512	16	EC34	512	128
EC14	1024	64	EC35	1024	256
EC15	1024	128	EC36	512	16
EC16	256	512	EC37	1024	256
EC17	1024	128	EC38	256	16
EC18	1024	1024	EC39	1024	64
EC19	512	32	EC40	1024	64
EC20	1024	32	EC41	512	64
EC21	1024	64			

MIC= Minimum inhibitory concentration; EC= *Escherichia coli*; KP= *Klebsiella pneumoniae*; CTX= Cefotaxime; CAZ= Ceftazidime

Table 2 Inhibition halos in millimeters of antimicrobials and ESBL

Strain isolate	CTX	CTX-CLA	CAZ	CAZ-CLA	ESBL
EC1	10	29	17	28	Yes
EC2	13	30	20	28	Yes
EC3	8	30	18	31	Yes
EC4	8	28	16	28	Yes
KP1	0	28	20	27	Yes
EC5	0	27	12	26	Yes
EC6	8	28	6	28	Yes

EC7	0	0	0	9	Yes
EC8	0	23	9	23	Yes
EC9	0	30	20	30	Yes
EC10	12	33	26	35	Yes
EC11	0	19	0	22	Yes
EC12	9	30	18	30	Yes
EC13	12	31	22	31	Yes
EC14	0	28	17	30	Yes
EC15	0	28	15	28	Yes
EC16	10	28	15	28	Yes
EC17	0	25	13	25	Yes
EC18	9	21	0	22	Yes
EC19	0	27	17	25	Yes
EC20	0	28	14	30	Yes
EC21	0	25	15	25	Yes
EC22	0	30	22	32	Yes
EC23	0	25	13	25	Yes
EC24	0	29	18	30	Yes
EC25	0	27	16	28	Yes
EC26	0	25	13	25	Yes
EC27	8	27	15	25	Yes
EC28	10	28	15	25	Yes
KP2	0	25	14	25	Yes
EC30	0	26	17	26	Yes
EC31	0	27	18	26	Yes
EC32	0	17	0	20	Yes
EC33	0	25	8	28	Yes
EC34	0	12	9	12	Yes
EC35	0	30	20	30	Yes
EC36	0	21	10	21	Yes
EC37	0	28	18	28	Yes
EC38	0	28	20	30	Yes
EC39	10	29	17	28	Yes
EC40	13	30	20	28	Yes
EC41	8	30	18	31	Yes

MIC= Minimum inhibitory concentration; EC= Escherichia coli; KP= *Klebsiella pneumoniae*; CTX= Cefotaxime; CAZ= Ceftazidime; CLA= Clavulanic acid

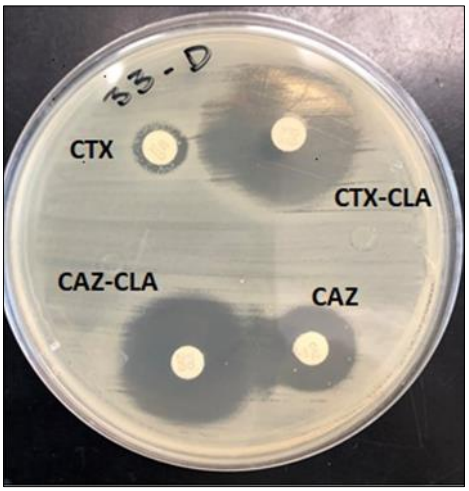

Figure 1 Detection of ESBL-producing strains by combined disc method. In the strain *Escherichia coli* EC33 a difference in the diameter of inhibition halos is observed between the antimicrobials CTX respect to CTX-CLA and with respect to CAZ-CLA

4. Discussion

More than 95% of UTIs are caused by *E. coli* which causes between 75-95% of the episodes of uncomplicated acute cystitis. In recent years, a progressive decrease in the sensitivity of this microorganism to the antimicrobials used has been detected [20]. It has been reported in Mexico and around the world that *E. coli* is the causative agent of UTIs which accounts for approximately 80 to 85% of the cases [21-25]. *E. coli* is a microorganism that belongs to the intestinal microbiota of the human organism, this pathogen could contaminate the rectum to the urethra, reaching spread to the bladder, and initiate infection at the time of excretion, affecting plus this pathogen to women. In the present study, *E. coli* was the most isolated followed by *K. Pneumoniae*.

In this study, it was detected that 100% of the strains were resistant to CTX and 95% of strains showed resistance to CAZ. This is analogous to previous studies showing the high percentage of resistance to CTX (88.9%) by *E. coli* strains (24). Since this study was focused on the strains, carrying ESBLs because they are a very important resistance mechanism in members of the Enterobacteriaceae family [26]. The dissemination of ESBLs has emerged to a high proportion of CTX-M enzymes, notably *E. coli*, which is now elevating in urinary tract infections [27,28]. In the present study, the 43 strains tested showed resistance to cephalosporins from third generation, CTX, and CAZ, it was possible to confirm that the 43 strains were phenotypically positive at the production of ESBL. It has recently been shown that infections with ESBL-producing bacteria represent a serious challenge for healthcare and are associated with higher mortality rates and healthcare-related costs [29]. The concern of bacterial resistance has increased in recent years; such is the case of this work where it is shown that the 43 strains tested presented a resistance of 100% for CTX and 95% for CAZ.

According to the results of the MIC, the 100% of strains showed high resistance to third-rate generation cephalosporin CTX, according to CLSI criteria and 88% of strains were capable of hydrolyzing CTX above the MIC value of ≥512 µg/mL. In contrast, for cephalosporin CAZ, 95% of strains showed resistance, whereas 11% of strains showed MIC value of ≥512 µg/mL. Suggesting that the ESBL type CTX-M is responsible for resistance since it is a genotype that is more abundant in the Enterobacteriaceae family, this genotype has as preferential substrates to CTX and CAZ. These resistance patterns were found to be similar to the earlier study conducted [30-33].

5. Conclusion

In this work, it was shown that the 43 study strains presented the same expression of ESBL for both cephalosporins, indicating that there are strains capable of inactivating these antimicrobials. The indiscriminate administration of antimicrobials, the lack of information on antibiotics, or the combination with other drugs has led to the generation of resistant bacterial strains, in this case, responsible for UTI, showing high resistance to β-lactams used in empirical treatment, manifested by the high MIC values obtained in this work.

E. coli is a bacillus gram-negative that is present in the human microbiota and is the most common uropathogen responsible for UTI, followed by *K. pneumoniae*. Both bacteria belong to the ESCAPE group (*Enterococcus faecium, Staphylococcus aureus, Clostridium, Acinetobacter baumannii, Pseudomonas aeruginosa*, and some enterobacterial) which are the leading cause of nosocomial infections throughout the world, it is necessary to insist on taking measures to achieve a reduction of these uropathogens both in the community and in the hospital, as well as being complemented with improvements in the hygienic and therapeutic management of patients suffering from bacterial infections.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

Author's contributions

All authors contributed equally to the conception and development of the work.

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