

Antibacterial Resistance Profile of *Enterobacterales* and Patient-Related Risk Factors in Northeast Algeria

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Abstract

In this retrospective study, performed between January 2020 and December 2021, three different clinical specimens' urine, blood, and pus from in and outpatients at Khenchela hospitals, North East of Algeria were analyzed. The profile of antibiotic susceptibility was characterized using the disk diffusion method. Out of the 1979 clinical specimens analyzed, 279 samples tested positive for bacterial infection with *Escherichia coli* as the most frequent strain isolated (about 55% of all cases). We found that all the isolated strains (*E. coli*, *Enterobacter* spp., *Klebsiella* spp., and *Proteus* spp.) were multidrug-resistant but showed high susceptibility to amoxicillin/clavulanic acid (29.7%). We found that *Enterobacter* spp. has a higher likelihood of developing resistance, it was 10.68-fold colistin-resistant, 3.6-fold imipenem resistant, 3.23-fold ciprofloxacin resistant, and 3.03-fold cefotaxime resistant more than the other groups. In contrast, *E. coli* exhibited the lowest resistance rates compared to all tested groups and was the most susceptible to the antibiotics evaluated. The logistic regression reveals that the variable "year" was significantly associated with changes in bacterial susceptibility patterns in three species *Klebsiella* spp., *Proteus* spp., and *E. coli* against four antibiotics ampicillin, ofloxacin, colistin, trimethoprim/sulfamethoxazole. For the variable "origin" the odds of resistance recorded were significant in *E. coli* against amoxicillin/clavulanic acid and imipenem and in *Klebsiella* sp. against amikacin, where antibiotic resistance is more relevant in outpatient. Finally, we revealed that gentamicin is less effective against *E. coli* for females than males (odds ratio = 0.33).

Keywords: *E. coli*, logistic regression, microbial resistance, multi-drug resistance, urinary tract infection.

Резюме

В това ретроспективно проучване, проведено между януари 2020 г. и декември 2021 г., бяха анализирани три различни клинични проби - урина, кръв и гной от хоспитализирани и амбулаторни пациенти в болниците в Кхенчела, Североизточен Алжир. Профилът на чувствителност към антибиотици беше характеризиран с помощта на метода на дискова дифузия. От 1979 анализирани клинични проби, 279 проби са дали положителен резултат за бактериална инфекция с *Escherichia coli* като най-често изолиран щам (около 55% от всички случаи). Установихме, че всички изолирани щамове (*E. coli*, *Enterobacter* spp., *Klebsiella* spp. и *Proteus* spp.) са мултирезистентни, но показват висока чувствителност към амоксицилин/клавуланова киселина (29.7%). Установихме, че *Enterobacter* spp. има по-висока вероятност за развитие на резистентност, като е 10.68 пъти по-резистентен към колистин, 3.6 пъти по-резистентен към имипенем, 3.23 пъти по-резистентен към ципрофлоксацин и 3.03 пъти по-резистентен към цефотаксим от другите групи. За разлика от това, *E. coli* показва най-ниски нива на резистентност в сравнение с всички тествани групи и е най-чувствителна към оценяваните антибиотици. Логистичната регресия показва, че променливата „година“ е значително свързана с промени в моделите на бактериална чувствителност при три вида *Klebsiella* spp., *Proteus* spp. и *E. coli* срещу четири антибиотика: ампицилин, офлоксацин, колистин, триметоприм/сулфаметоксазол. За променливата „произход“ регистрираните вероятности за резистентност са значителни при *E. coli* срещу амоксицилин/клавуланова киселина и имипенем и при *Klebsiella* sp. срещу амикацин, където антибиотичната резистентност е по-значима при амбулаторни пациенти.

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Acta Microbiol. Bulg. 2025; 41(02). <https://doi.org/10.59393/amb25410205>

Накрая, разкрихме, че гентамицинът е по-малко ефективен срещу *E. coli* при жените, отколкото при мъжете (коефициент на вероятност = 0.33).

Introduction

The alarming pattern of antimicrobial resistance observed in many parts of the world becomes a major public health problem because of the inappropriate use of antibiotics, and the adoption of empirical treatment without knowing the bacteriological profile of the patients. This emerging threat causes often treatment failure of infections augments the medical burdens and the mortality and morbidity levels (Boubendir and Beldi, 2018). Indeed, in recent years, there has been a significant increase in antibiotic resistance, particularly among Gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*. Resistance to β -lactams in enterobacteria is dominated by the production of Extended-Spectrum Beta-Lactamases (ESBL). The strains producing these enzymes are often the cause of potentially serious infections in both hospital and community settings (Arlet, 2014; Ait-Mimoune *et al.*, 2022).

The persistent exposure of bacterial strains to antibiotics is a major contributor to the development of antibiotic resistance, but there are also many patient-related factors that can contribute (Sahuquillo-Arce, 2011; Koložsvári *et al.*, 2019). For instance, patients who have been recently hospitalized, who have had recent medical procedures, or who live in long-term care facilities are at increased risk for antibiotic resistance due to increased exposure to antibiotics. It is largely admitted that the burden of antibiotic resistance continues to increase among patients in hospitals (Mulvey and Simor, 2009). For example, infections caused by multiple resistant strains such as *K. pneumoniae* and *Pseudomonas aeruginosa* often follow intense antibiotic use in many hospitals (Struelens, 1998). Gender can influence every part of an individual's health; many studies have shown conflicting results regarding the susceptibility of women and men to antibiotic-resistant infections. Some studies have found that women may be more susceptible to certain types of antibiotic-resistant infections (Jones *et al.*, 2022), while others have found that men have a higher incidence rate of infections with antimicrobial-resistant pathogens (Brandl *et al.*, 2021), especially of respiratory tract infections (Falagas *et al.*, 2007). The exact mechanisms underlying these differences are not well understood and need more research to be determined. Age can also impact a person's susceptibility to infection, elderly and young children are at increased risk of antibiotic re-

sistance due to decreased immunity and increased exposure to antibiotics, it was estimated that up to 30% of deaths in the world are caused by multidrug-resistant bacterial infections are newborns (Romandini *et al.*, 2021). Additionally, community-acquired infections may also contribute to the spread of antibiotic-resistant bacteria. Thus patient-related factors such as sample origin, sex, age, and hospital or community patient must be taken into consideration at the moment of empirical prescription of antimicrobials (Linhares *et al.*, 2013).

While there has been significant research on antibiotic resistance in recent years, there are still many unanswered questions about the specific risk factors that contribute to the development of antibiotic-resistant bacteria. While exposure to antibiotics is a major contributor to the development of antibiotic resistance, there is still much to be learned about how other host-dependent factors may influence the development of resistance. Further research is needed to better understand the complex interactions between these factors and to identify new strategies for preventing and controlling antibiotic resistance. Therefore, the aim of this paper is to investigate the susceptibility patterns of bacterial strains isolated from different clinical specimens at Khenchela hospitals, North East of Algeria, according to culture site and patient age, gender, samples originating from hospital-admitted patients versus samples from the community, and to study the possible relationships observed. This data allows us to understand the importance of the risk of the circulation of multi-resistant bacteria on a national scale.

Materials and Methods

Study Design

This is a retrospective study, performed between January 2020 and December 2021, conducted at Microbiology Laboratories at Khenchela hospitals (Ahmed Ben Bella and Hihi Abdelmadjid), North East of Algeria. The population included all in and out untreated patients with different clinical symptoms of microbial infections. Three different clinical specimens were analyzed, urine, blood, and pus.

Sampling and isolation

In the case of adults, clean catch midstream urine samples were collected in sterile disposable containers (20 ml) with tight-fitting lids to prevent leakage, while in the case of children aged less than 3 years, adhesive urine collection bags were used

for specimen collection. The samples were analyzed in a period not exceeding 2 hours in order to avoid the overgrowth of bacteria in the urine. The collected urine specimens were subjected to general urine examinations using direct microscopy for white blood cell counting, then cultured on Nutrient agar, Hektoen agar, and Chapman agar (bioMérieux, France), and incubated aerobically for 24 hours at 37°C. A specimen was considered positive for UTI in light of the number of yielded colonies ($\geq 10^5$ cfu/mL) (Moinard *et al.*, 1987). Blood samples were introduced into special bottles containing a nutrient broth (bioMérieux, France) and an anticoagulant, and, in many cases, resins or charcoal mixtures to reduce the effects of antimicrobials and other toxic compounds. Aspirated blood culture fluid was Gram-stained for preliminary identification and inoculated on Columbia blood agar and Chocolate agar (bioMérieux, France). All solid media were incubated at 37°C in 5% CO₂ for up to 5 days (Kirn and Weinstein, 2013). Pus samples were aseptically collected using a sterile swab in a test tube and inoculated onto Blood agar, Hektoen agar, and Chapman agar (bioMérieux, France). Plates were incubated at 37°C for 24 hours (Sathish *et al.*, 2020).

Bacterial identification

Bacterial identification was based on standard culture and biochemical characteristics of isolates. Gram-negative bacteria were identified by standard biochemical tests (Api 20E, bio Mérieux, France). Gram-positive isolates were stained and observed under a microscope to confirm their Gram and highlight their shape (cocci or bacilli). Gram-positive cocci include *Staphylococcus* (catalase-positive), which grows in clusters, and *Streptococcus* (catalase-negative), which grows in chains. Gram-positive bacilli (rods) subdivide according to their ability to produce spores. *Bacillus* and *Clostridia* are spore-forming rods while *Listeria* and *Corynebacterium* are not (Bonadio *et al.*, 2001).

Antibiotic susceptibility testing

Overall, 279 bacterial strains were isolated from positive cultures and tested for their susceptibility to antibiotics. The antibiogram was carried out by the disk diffusion method on Muller–Hinton agar (bioMérieux, France), according to the recommendations of the CLSI guidelines (Wayne, 2020). The following antibacterial agents were used: penicillin G (P) (6 µg), ampicillin (10 µg), amoxicillin + clavulanic acid (30 µg), cefazolin (30 µg), cefotaxime (30 µg), cefazolin (30 µg), imipenem (10 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), ofloxacin (15 µg), and colistin (10 µg). Commercial antibiotic disks were purchased from Bio-Rad (France) and Oxoid (England). The plates were incubated for 24 h at 37°C and inhibition zones were measured. The results were recorded as resistant, intermediate, or susceptible by the measurement of the inhibition zone diameter according to the interpretive standards of CLSI. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains.

cin (5 µg), ofloxacin (15 µg), and colistin (10 µg). Commercial antibiotic disks were purchased from Bio-Rad (France) and Oxoid (England). The plates were incubated for 24 h at 37°C and inhibition zones were measured. The results were recorded as resistant, intermediate, or susceptible by the measurement of the inhibition zone diameter according to the interpretive standards of CLSI. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains.

Statistical analysis

A chi-square test for independence was used to test if there is a relation between positive results and (1) the season of samples recovering, and (2) the type of biological fluid sample. Significance was assumed at a p-value of <0.05.

Multivariate logistic regression models were performed on the patient-related data and the antimicrobial resistance. The patient-related factors (independent variables) were (1) year (2020 vs 2021), origin (inpatient: refers to a patient who is admitted to a hospital or other healthcare facility and requires an overnight stay vs outpatient: a patient who does not require an overnight stay in a hospital or other healthcare facility), sex (male vs female). The differences were quantified with odds ratios (OR), applying 95% confidence intervals. The computations were performed with R Core Team (2021).

Results

Bacterial infections

A total of 1979 clinical specimens obtained from patients suspected of bacterial infections were analyzed from January to December 2021. There were 279 positive cultures with a percentage of 14 % and 1700 negative cultures (86%). Statistically, there was no significant difference between seasons according to the distribution of positive and negative cases (X-squared = 0.22, df = 3, p-value = 0.97) (Fig. 1).

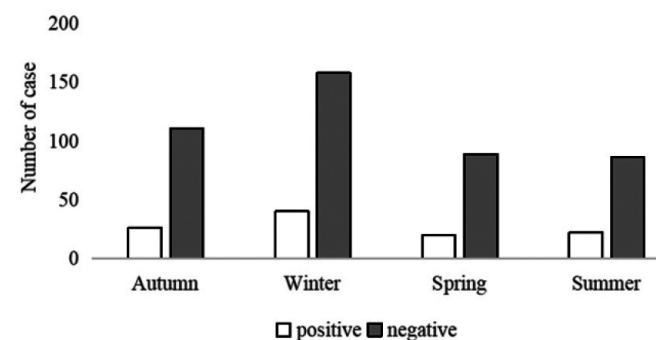


Fig. 1. Distribution of positive and negative cultures across seasons

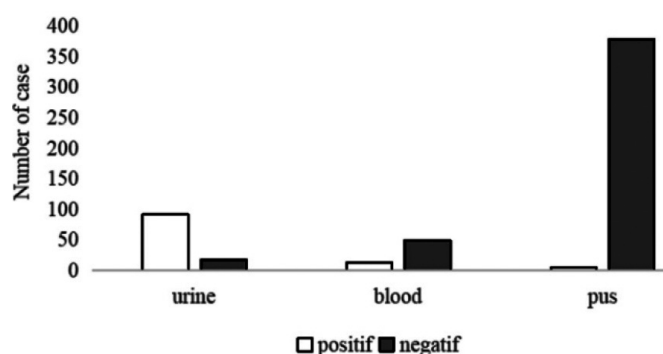


Fig. 2. Cultures positive prevalence across the three different body fluids analyzed

Among the three sources of samples, there was a high significant difference (X-squared = 368.37, df = 2, p-value < 0.01) according to the distribution of positive and negative cases (Fig. 2). The highest frequency (84%) was unregistered for urine-positive cultures followed by blood cultures (11%), and pus had the lowest cultures positive prevalence (4%).

Bacterial species

Among the studied population we found that *E. coli* was the most frequent strains isolated, account for about 55%, followed by *Enterobacter* spp. with 25% of all cases (Fig. 3). The other bacterial species are less common and account for less than 15% of all cases of bacterial infections.

Antimicrobial resistance

Among the four groups of isolates tested, multidrug resistance was commonly observed, appearing in all groups (Table 1). Resistance profiles against (5 antibiotics) amoxicillin/clavulanic acid, cefazolin, amikacin, ofloxacin, and trimethoprim/

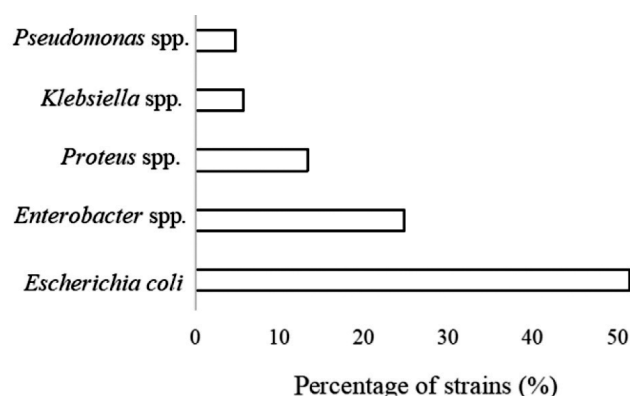


Fig. 3. Percentage of isolated bacteria from the different biological fluid cultures of the patients

sulfamethoxazole are similar in the four bacterial species (p-value under chi-square test > 0.05) (Table 1). Resistance was particularly lower (< 25%) in *E. coli* and *Enterobacter* spp. against amoxicillin/clavulanic acid, *Enterobacter* spp. against ofloxacin, and *Proteus* spp. against colistin. In contrast, *Klebsiella* spp. present the higher resistance (81.48) against ampicillin.

More specifically and in order to allow a statistically robust assessment of the collected data, the resistance differences are compared between strains of the four isolated genera. The odds ratio (logistic regression) that the three groups would differ from *E. coli* (as reference) was calculated, along with the p-value, to determine whether any of the differences between the four groups were significant. For clarity, only odds ratios of significant differences are included (Table 2). We found that the *Enterobacter* spp. has the highest likelihood of developing resistance, it was 10.68-fold colis-

Table 1. Resistance profile of all isolates (bold character not significant)

Antibiotic	% resistant					Chi-Square Test (p-value)
	All	<i>Enterobacter</i> spp.	<i>Klebsiella</i> spp.	<i>Proteus</i> spp.	<i>Escherichia coli</i>	
Ampicillin	49.8	33.34	81.48	50	47.36	< 0.05
Amoxycillin/ clavulanic acid	29.7	22	45.5	34.8	25	0.219
Cefazolin	31.9	33.3	37.5	26.3	31.3	0.12
Cefotaxime	43.9	56.4	55.2	53.8	29.9	< 0.05
Imipenem	52.5	77.3	42.1	50	48.7	< 0.05
Gentamicin	57.1	77.8	46.7	56	51.9	< 0.05
Amikacin	52.5	64.3	37	53.8	48.7	0.08
Ciprofloxacin	42.9	70.4	36.8	52.6	18.2	< 0.05
Ofloxacin	40.9	25	58.3	34.8	43.5	0.11
Colistin	65.8	68.4	84.2	22.2	70.1	< 0.05
Trimethoprim/ sulfamethoxazole	62.9	58.1	55.2	69.6	65.3	0.63

tin-resistant, 3.6-fold imipenem resistant, 3.23-fold ciprofloxacin resistant, and 3.03-fold cefotaxime resistant more than the other groups. Followed by *Klebsiella* spp. have a higher likelihood of 4.9-fold Cefotaxime resistance than the other genera.

Then the *Proteus* spp. was the more resistant (5-fold) to ciprofloxacin, and the less resistant to (-0.5) to colistin. Finally, the prevalence of antimicrobial resistance (resistance to almost all antibiotics except colistin) of *E. coli* seemed to be the lowest compared to all tested groups.

Patient-related factors and antimicrobial resistance

The results of the logistic regression (Table 3) indicate that the “year” variable is significantly associated with changes in the susceptibility patterns of three bacterial species (*Klebsiella* spp., *Proteus* spp., and *E. coli*) against four antibiotics (ampicillin, ofloxacin, colistin, trimethoprim/sulfamethoxazole). *Klebsiella* spp. had the highest odds ratio to be resistant to ampicillin 25 times in 2021 than in 2020, followed by *Proteus* spp. and *E. coli*. The susceptibility profile of *Enterobacter* spp. remains unchangeable. The variable “origin” also influences the likelihood of being resistant in *E. coli* against amoxicillin/clavulanic acid (OR= 0.08) and imipenem (OR = 0.11), it seems that strains steaming from outpatient resist these two antibiotics more than those from inpatient. Concerning the variable “sex” the odds (OR= 0.33) of resistance recorded

were only significant in *E. coli* against gentamicin, where antibiotic resistance is more relevant in females.

Discussion

This study aims to describe the susceptibility patterns of bacterial strains isolated from different clinical specimens at Khenchela hospitals, North-east of Algeria, and to identify the relationships between susceptibility patterns and various patient characteristics, such as age, gender, culture site, and hospital vs community origin. This data will provide insight into the risk of the circulation of multi-resistant bacteria on a national scale and can inform public health strategies aimed at preventing the spread of antibiotic-resistant infections. The results of this study may help to identify specific populations that are at higher risk for antibiotic-resistant infections and to develop targeted interventions to reduce the spread of these infections.

According to the distribution of positive and negative cases in the studied population, we did not find evidence of a clear pattern or relationship with the seasons. Our results may indicate that bacterial infections do not have a strong seasonal component, or that other factors are more influential in determining its spread. This statement is in line with the basic assumption that bacterial infections do not discriminate based on calendars or dates, and can affect people regardless of time (Linhares *et al.*, 2013). Further research and analysis are needed to

Table 2. Statistically (logistic regression) significant difference between strains of the four isolated genera

Species	Ampicillin			Cefotaxime			Imipenem		
	N total	N resistant	OR (94% CI)	N total	N resistant	OR (94% CI)	N total	N resistant	OR (94% CI)
<i>Escherichia coli</i>	114	54	Reference	77	23	Reference	78	38	Reference
<i>Enterobacter</i> spp.	36	12	NS	39	22	3.03 (1.36-6.75)	22	17	3.6(1.2-10.66)
<i>Klebsiella</i> spp.	27	22	4.9 (1.7-13.8)	29	16	2.9 (1.19-6.96)	19	8	NS
<i>Proteus</i> spp.	24	12	NS	26	14	2.8 (1.1-6.82)	22	11	NS
Species	Gentamicin			Ciprofloxacin			Colistin		
	N total	N resistant	OR (94% CI)	N total	N resistant	OR (94% CI)	N total	N resistant	OR (94% CI)
<i>Escherichia coli</i>	77	40	Reference	33	6	Reference	77	54	Reference
<i>Enterobacter</i> spp.	36	28	3.23 (1.31-7.9)	27	19	10.68 (4-35.8)	38	26	NS
<i>Klebsiella</i> spp.	30	14	NS	19	7	NS	19	16	NS
<i>Proteus</i> spp.	25	14	NS	19	10	5 (1.4-17.6)	18	4	(-) 0.5 (0.03-0.41)

OR: odds ratio, N: number of strains, CI: confidence interval at 95%; NA: not significant, (-) decreased resistance

Table 3. Multivariate logistic regression models on the patient-related variables and antimicrobial resistance of the *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp. and *E.coli*

ATB	Variables	Strains							
		<i>Enterobacter</i> spp.		<i>Klebsiella</i> spp.		<i>Proteus</i> spp.		<i>Escherichia coli</i>	
		odds	p-value	odds	p-value	odds	p-value	odds	p-value
Ampicillin				Year (2) 25.33 (2.69-604.19)	0.01	Year (2) 7.00 (1.18-60.52)	0.04	Year (2) 3.04 (1.42-6.69)	0.004
Amoxycillin/ clavulanic acid	Year	Ns	Ns	Ns	Ns	Ns	Ns	Origin (2) 0.08 (0.004-0.67)	0.039
Imipenem	Origin	Ns	Ns	Ns	Ns	Ns	Ns	Origin (2) 0.11 (0.03-0.34)	0.00025
Gentamicin	Sex (female vs male)	Ns	Ns	Ns	Ns	Ns	Ns	Sex (1) 0.33 (0.10-0.94)	0.039
Amikacin	Origin	Ns	Ns	Origin (2) 0.041 (0.0009-0.61)	0.04	Ns	Ns	Ns	Ns
Ofloxacin	Year	Ns	Ns	Ns	Ns	Ns	Ns	Year (2) 0.08 (0.02-0.25)	< 0.001
Colistin	Year	Ns	Ns	Ns	Ns	Ns	Ns	Year (2) 0.06 (0.01-0.26)	< 0.001
Trimethoprim/ sulfamethoxazole	Year	Ns	Ns	Year (2) 12.5 (1.57-156.7)	0.02	Ns	Ns	Year (2) 0.2 (0.08-0.44)	<0.01

Were year : 2020 (1) vs 2021(2), origin: inpatients (1) vs outpatients(2), and sex : female (1) vs male (2)

gain a better understanding of their incidence and changes in factors associated with the diseases.

Our results showed that urinary tract infections (UTIs) were the most frequently occurring type of infection. Indeed, UTIs are one of the most frequent infectious diseases worldwide (Hryniewicz, 2001; Khoshbakht *et al.*, 2013). It was among the top three most common bacterial infections experienced by humans, with respiratory and gastrointestinal infections being more prevalent (Najar *et al.*, 2009). These types of infections are caused by bacteria, with *E. coli* being the most common one (Foxman, 2010) which is in agreement with our results when *E. coli* was the most frequent strain isolated followed by *Enterobacter* spp., *Proteus* spp., *Klebsiella* spp. and finally *Pseudomonas* spp. The predominance of gram-negative isolates mainly *E. coli* has been also reported in many studies worldwide (Aly and Balkhy, 2012; Zhanel *et al.*, 2013;

Farah *et al.*, 2019). This supports the conclusion that *E. coli* continues to be the most common cause of community-acquired UTI due to the fact that it belongs to the normal flora of the human intestine and therefore easily colonizes the urinary tract (Céu Costa *et al.*, 2009).

In this study, four bacterial species were tested (*E. coli*, *Enterobacter* spp., *Klebsiella* spp., and *Proteus* spp.) for antibiotic resistance. The results showed that all of them were multidrug-resistant, as they exhibited resistance to at least one antimicrobial drug in three or more antimicrobial categories (amikacin, ofloxacin, and trimethoprim/sulfamethoxazole) (Magiorakos *et al.*, 2012). The tested microorganism species behave similarly against five antibiotics, amoxicillin/clavulanic acid, cefazolin, amikacin, ofloxacin, and trimethoprim/sulfamethoxazole. All of them were susceptible to amoxicillin/clavulanic acid and cefazolin and have in oppo-

site a higher resistance to amikacin, ofloxacin, and trimethoprim/sulfamethoxazole. Interestingly, these results contradict the findings of a previous local and regional study conducted respectively at Guelma district 160 km away from our study region, Morocco and Tunisia (Bouzenoune *et al.*, 2009; Nadmi *et al.*, 2010; Bentroki *et al.*, 2012), where this group of microorganisms was found to be highly resistant to amoxicillin/clavulanic acid, cefazolin. On the other hand, our findings confirm that amoxicillin-clavulanic acid and cefazolin continue to be active against pathogens as reported in (Cormio *et al.*, 2003). This clearly shows that the prevalence of antibiotic resistance between different regions depends on differences in populations of the served patients such as hygiene practices, antimicrobial use and misuse, strategies of hospital infection control, living in an environment with resistant bacteria, the level of crowding in the community, etc. (Bruinsma *et al.*, 2003; Alhumaid *et al.*, 2021). Such information is important because it provides context for the study and helps to determine the geographic scope of the research. Context-specific epidemiological studies are important to gain a deeper understanding of the spread of antimicrobial resistance (AMR) in different populations. It can then be used to inform targeted intervention strategies that are effective in reducing the burden of AMR in those populations (Allcock *et al.*, 2017).

Our data analysis regarding the susceptibility patterns of antimicrobials provides a statistically robust assessment of the resistant levels in the four isolated genera. We found that *Enterobacter* spp. has a higher probability of being resistant, mainly against colistin, imipenem, ciprofloxacin, and cefotaxime more than the other groups. Followed by *Klebsiella* spp. that have a higher likelihood of cefotaxime resistance and *Proteus* spp. was the more resistant to ciprofloxacin. The resistance mechanisms in these *Enterobacterales* species are diverse, often involving a combination of intrinsic and acquired factors. Studies indicate that a significant proportion of these bacteria exhibit fluoroquinolone resistance, frequently co-occurring with extended-spectrum β -lactamase (ESBL) production. The co-existence of β -lactam and fluoroquinolone resistance markers is a major concern in antimicrobial resistance, particularly in Gram-negative bacteria. Research has shown that various resistance genes can be present within the same bacterial isolates, highlighting the complex interplay between different resistance mechanisms (Egwu *et al.*, 2023; Kawa *et al.*, 2023). This co-existence poses a serious risk for treatment

failure and complicates infection management, especially in vulnerable populations such as renal transplant recipients (Truşcă *et al.*, 2017). Indeed, the ESKAPE group, which stands for *Enterococcus faecium*, *Staphylococcus aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species are considered particularly problematic because they are often resistant to multiple antibiotics (Davin-Regli *et al.*, 2019). Also, these pathogens are often associated with hospital-acquired infections and are responsible for a high risk of mortality (Founou *et al.*, 2017). Throughout this study, we reveal the ineffectiveness of the aforementioned antibiotics against ESKAPE and accordingly, it should be removed from the antibiotic lists suggested against this group of pathogens in the region. It is, therefore, important to find alternative ways to treat infections, particularly those caused by ESKAPE pathogens. For *E. coli* the situation remains relatively reassuring since it was the lowest resistant bacteria, it was highly susceptible to ciprofloxacin and amoxicillin/clavulanic, cefotaxime, and cefazolin. Despite the low percentages of resistance against the precedent antibiotics compared to other studies worldwide (Ketani Halabi *et al.*, 2021), it is important to mention here that such a statement was only proprietor to the sampled population. For example, some strains of *E. coli* have developed resistance to fluoroquinolones, a class of antibiotics commonly used to treat urinary tract infections, and extended-spectrum β -lactamase (ESBL) producing *E. coli* are resistant to many commonly used antibiotics, including penicillin and cephalosporins (Yadav *et al.*, 2015). We should therefore be cautious when making statements about the resistance patterns of different bacteria, as resistance rates can vary widely and are constantly evolving. It is always best to consult with a healthcare professional to determine the most appropriate treatment options based on the specific circumstances of each case.

In this study, we have examined the relationships between these susceptibility patterns and various patient characteristics, in order to provide a comprehensive picture of the current state of bacterial susceptibility patterns in the studied population. The logistic regression reveals that the variable “year” was significantly associated with changes in bacterial susceptibility patterns in three species *Klebsiella* spp., *Proteus* spp., and *E. coli* against four antibiotics Ampicillin, Ofloxacin, Colistin, Trimethoprim/sulfamethoxazole. Internationally, the number of antibiotics effective against ESKAPE is

decreasing with every passing year (Mulani *et al.*, 2019). The comparison between the two years of the study showed an increase in the emergence of antimicrobial resistance, particularly against ampicillin. The odds of resistance recorded were 25 times higher in *Klebsiella* spp., 7 times higher in *Proteus* spp., and 3 times higher in *E. coli* in 2021 compared to 2020. Ampicillin was originally a first-line drug for treatment, but its widespread use has led to many microorganisms becoming resistant to it (Swami, 2014).

The 2020 corresponds to the peak of the COVID-19 pandemic, many experts assumed that indiscriminate use of antibiotics, 72% of COVID-19 patients got different types of antibiotics where Ampicillin was the most prescribed during this period might reduce the effectiveness of some antibiotics, and potentially develop AMR that might be the next global health pandemic (Rawson *et al.*, 2020; Mahadi, 2021). The second patient-related factor we revealed also associated with changes in bacterial susceptibility patterns was origin (inpatients vs outpatients). In contrast to the common perception that inpatients are more likely to be affected by antibiotic resistance and the rates of antibiotic-resistant infections have become increasingly commonplace in hospitalizations during the past decade (Cosgrove and Carmeli, 2003; Mainous *et al.*, 2011), we found that antibiotic resistance is more relevant in outpatient mainly in *E. coli* against amoxicillin/clavulanic acid and imipenem, and in *Klebsiella* sp. against amikacin. The development of *E. coli* resistant to Amoxycillin/clavulanic acid is particularly worrisome because this antibiotic is a key drug in the armamentarium used in the outpatient setting (Akpoji *et al.*, 2022). In general, the finding that antibiotic resistance is more frequent in outpatients than inpatients is concerning. This is likely due to a number of factors, including the overuse of antibiotics in the community and the limited availability of alternative treatments. In addition, outpatients may also be less likely to complete a full course of antibiotics, which can contribute to the development of resistance. It is therefore important to monitor the prevalence of antibiotic resistance in both inpatient and outpatient populations to identify trends and take action as needed. Lastly, we found that gender can influence the bacterial susceptibility patterns but only in *E. coli* against gentamicin when male uropathogens present a lower susceptibility to this antibiotic than those isolated from females.

Many studies also sex-related differences in antimicrobial resistance (Gu *et al.*, 2022). For exam-

ple, in a ten-year surveillance study (2000–2009) in Portugal and a demographic study on antibiotic resistance in India *E. coli* isolated from males showed resistance to the majority of antibiotics (Linhares *et al.*, 2013; Chatterjee *et al.*, 2014). Men and women may have different risk factors for acquiring antibiotic-resistant infections, such as differences in healthcare-seeking behavior or exposure to different sources of antibiotics. Thus, to avoid multi-drug-resistant bacteria increase antibiotics should be used empirically according to gender.

Besides the study limitations (relatively small sample size, a specific geographic location, and limited period) we believe that our findings can be valuable for public health officials and healthcare providers, as they can inform the development of targeted interventions aimed at reducing the spread of antibiotic-resistant infections and improving public health outcomes.

Conclusions

The study focused on the epidemiology of antibiotic resistance, rather than the underlying mechanisms of resistance. Throughout this study, we confirm that urinary tract infections were the most frequently occurring type of infection with *E. coli* as the most causal agent. We found that all isolated strains were multidrug-resistant, with *E. coli* exhibiting the highest susceptibility to antibiotics among the tested bacteria. We also reported that some patient-related factors may be associated with an increased risk of antibiotic resistance. Firstly, the prevalence of resistant pathogens increased over time, particularly after the peak of the COVID-19 pandemic. This highlights the need for continued surveillance of antibiotic resistance patterns in the context of pandemics, as changes in healthcare access and infection control practices during these events may have an impact on the spread of resistant pathogens. Secondly and interestingly, we found that antibiotic resistance is more relevant in outpatient particularly for amoxicillin/clavulanic acid leading thereby to raise our awareness to monitor the prevalence of antibiotic resistance in both inpatient and outpatient populations. And finally, we revealed that Gentamicin is more effective against *E. coli* for males than females. Further research is needed to understand the reasons behind this difference and whether it has any implications for the development and spread of antibiotic resistance.

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