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ISSN: (3065- 1786)

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https://multisciajournals.com/journals/index.php/fp

Frontiers in Pharmaceutics
Volume 1 Issue 2 2025

Various clinical isolates' profiles of antimicrobial resistance to third-generation cephalosporins

K Purushotham
Department of **Pharmaceutics**

Article Info

Received: 24-02-2025 Revised:22-03-2025 Accepted:09-04-2025 Published:21-04-2025

1. Introduction

One of the biggest threats to global public health in the twenty-first century is antimicrobial resistance (AMR) [1]. The geographical regions impacted by medication resistance, the quantity of resistant microbial strains, and the degree of resistance in each organism are all increasing [2]. Furthermore, the proportion of organisms displaying AMR—particularly resistance to several antibiotics—is steadily rising [3]. As a result, diseases that were previously believed to be amenable to medications are now resurfacing in new, antibiotic-resistant categories [4].

Because they raise the possibility of inappropriate treatment, resistant bacteria increase morbidity and mortality [5, 6]. Treatment may be delayed and hampered by this resistance, leading to problems or even death [7, 8]. Additionally, a patient might require more care and the use of more costly and different medications, which might have more serious side effects, or they might require more invasive hospital treatments such intravenous injections [6, 9].

Multi-resistant organisms make treatment more difficult, expensive, and occasionally ineffective. Multidrug resistance (MDR) diseases can kill people. due to the failure of every medication now on the market, particularly in underdeveloped nations [10]. For example, public health in underdeveloped nations has been jeopardized by MDR enteric disease pathogens [3]. Mycobacterium tuberculosis, Enterococcus fecium, Enterobacter cloacae, Klebsiella pneumoniae, Staphylococcus aureus, Acinetobacter baumanii, and Pseudomonas aeruginosa have all been found to have MDR worldwide [11].

According to the clinician's prior clinical experience, many infections could be successfully treated in the past (i.e., empirical therapy) [12, 13]. However, as resistance has been found to almost every antimicrobial drug now authorized for use in human and veterinary clinical treatment, this technique is becoming more than the exception to the rule. This, together with the wide range of antimicrobial agents that are now on the market, makes choosing the right agent more difficult. This condition emphasizes the significance of the diagnostic laboratory in clinical practice and has increased the reliance of doctors on findings from in vitro antibiotic susceptibility testing [14].

The appropriate course of action for each patient is determined by data on AMR among local pathogens [15, 16]. However, the percentage of resistant bacteria might differ between regions [17], and local information on resistance trends is lacking in many medical facilities [18]. Data, when available, can be used for a variety of purposes, such as guiding treatment decisions, comprehending AMR trends, influencing public health policy, identifying priority areas for interventions, and tracking the effectiveness of interventions to contain resistance, according to experiences from surveillance networks on antimicrobial use and AMR [1]. However, information on the profile of drug antimicrobial resistance is lacking, particularly in underdeveloped nations like Ethiopia. Thus, screening for antimicrobial resistance is a part of the current work.

characteristics of the third-generation cephalosporin medications used at Jimma University Specialized Teaching Hospital to treat infectious illnesses.

2. Supplies and Procedures

2.1. Specimen collection and study design. From April to August 2016, Jimma University Specialized Hospital (JUSH) hosted a hospital-based cross-sectional study. The hospital was chosen because it offers a wide range of services for people from all over the nation with a variety of health issues. Trained nurses gathered clinical samples from hospitalized patients, including stool, sputum, urine, and wound swabs.

2.2. Identification of bacteria. Every clinical sample was gathered using a conventional microbiological procedure in order to identify and isolate harmful germs. Each specimen was then plated onto MacConkey agar, Blood agar, Mannitol Salt agar, Xylose lysine deoxycholate agar, Chocolate agar, and Thayer-Martin agar, depending on the source of the samples, and incubated aerobically at 37°C for 24 hours. Clusters of gram-positive cocci that are both catalase and coagulase positive and have a distinctive yellow to golden hue

Mannitol fermentation on MSA in conjunction with blood agar colonies was used to distinguish Staphylococcus aureus from other gram-positive cocci. Standard microbiological algorithms, such as Gram's stain (gram-negative bipolarly stained bacilli for Yersinia spp.), colonial growth characteristics, and appearance on enriched and selective media were used to identify the gram-negative bacilli, coliforms, Proteus species, and Yersinia enterocolitica. These methods were combined with standard biochemical tests described in the reference material [19]. The clinical isolates in question and of clinical significance were identified using biochemical tests such as lactose, glucose, and sucrose fermentation with and without H2S production (using TSI/KIA), lysine decarboxylation (LDC), indole and citrate utilization (MIS), methyl red (MR), Voges-Proskauer (VP), and pyrrolidonyl aminopeptidase (PYR) [19, 20]. As a result, from the clinical samples that were gathered, clinical strains of Yersinia enterocolitica, Escherichia coli, Klebsiella pneumoniae, Proteus species, Citrobacter freundii, Citrobacter Koseri, Enterobacter cloacae, Klebsiella oxytoca, and Enterobacter aerogenes were isolated.

2.3. Testing for antimicrobial susceptibility. S. aureus ATCC 25923 and quality control strains were used in antimicrobial susceptibility testing employing disk diffusion technique in accordance with the Kirby-Bauer method [21]. As a result, from an agar plate culture, three to five well-isolated colonies of the same morphological type were chosen, moved into Muller Hinton broth, and cultured for twenty-four hours at 37°C. Sterile saline was used to modify the suspension's turbidity until it was visually equivalent to the 0.5 McFarland criteria. The freshly made Mueller Hinton agar plate was then streaked with the swab throughout its whole surface. Within fifteen minutes of inoculation, the antimicrobial disks were placed on the plates. After that, the plates were incubated for 24 hours at 37°C. Based on resistance data interpreted in accordance with the Clinical and Laboratory Standards Institute [22], a zone of inhibition was assessed, and the results were classified as sensitive, resistant, or intermediate. Third-generation cephalosporins, ceftriaxone (30 μg) and ceftazidime (CAZ) (30 μg), were the antibacterial drugs that were examined. Additionally, MDR profiles for strains resistant to cephalosporin medications were identified against various antimicrobial classes, including ciprofloxacin (5 μg), sulfamethoxazole-trimethoprim (25 μg), amikacin (AMK) (30 μg), piperacillin (PIP) (100 μg), amox-clavulanic acid (AUG), and ciprofloxacin (CPR) (5 μg). Abtek Biologicals Ltd., Liverpool L9 7AR, UK, produced all of the antibiotic discs that were utilized.

2.4. Quality Management. Throughout the entire laboratory work process, quality control methods were implemented to ensure the reliability of the study findings. Prior to usage, the typical shelf life of culture

media, antibiotic discs, and staining reagents was examined. After being prepared and sterilized by autoclaving at 121°C for 15 minutes, all culture plates and antibiotic discs were kept at the prescribed refrigerator temperature. The norm

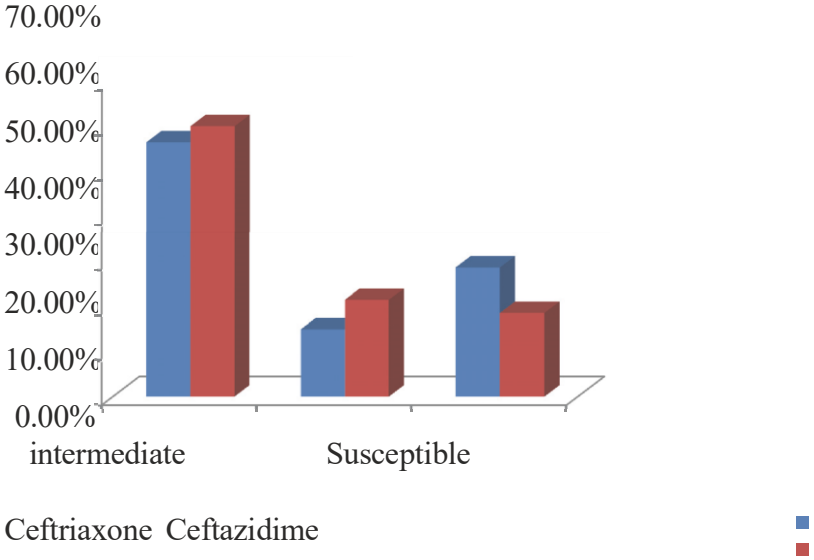
ABLE 1: Distribution of isolates in clinical specimens collected from patients.

Clinical isolates	Specimen type				
	Sputum	Urine	Wound Swab	Stool	**Total**
Escherichia coli	-	29	3	31	63
Citrobacter spp.	3	11	25	12	51
Enterobacter species	16	13	15	-	44
Klebsiella oxytoca	-	-	6	-	6
Klebsiella pneumonia	23	-	-	3	26
Staphylococcus aureus	6	3	38	-	47
Proteus species	-	-	5	-	5
Yersinia enterocolitica	3	-	3	-	3
Total	51	56	95	46	248

reference bacterial strains were tested as a positive control on the biochemical tests and agar plates with antibiotic discs. Proper sample collection and handling were done by experienced nurses who were working at each ward unit.

1.1. Data Analysis. Data were edited, cleaned, entered, and analyzed using statistical package for social science (SPSS) version 16. Descriptive analysis such as frequencies and mean were used. P value of < 0.05 was considered to indicate statis- tically significant differences and the results were presented using tables and figure.

2. Results



About 388 clinical specimens were collected from sputum, urine, wound swab, and stool of hospitalized patients hav- ing clinically evident infection (patients with complaints of urinary tract infection, open wounds, pneumonia, and upper respiratory tract infections). Totally, 248 (64%) bac- terial isolates were obtained from 154 (62.1%) male and 94 (37.9%) female study subjects. In the present study *Escherichia coli* (25.4%) and *Staphylococcus aureus* (19.0 %) were the predominant organisms isolated from the study subjects. The other bacterial isolates include *Citrobacter freundii* (12.1%), *Citrobacter koseri* (8.5%), *Enterobacter cloacae* (13.0%), *Kleb- siella oxytoca* (2.4%), *Klebsiella pneumoniae* (10.5%), *Enter- obacter aerogenes* (4.8%), *Proteus* species (2.0%), and *Yersinia enterocolitica* (2.4%) as indicated in Table 1.

All the bacterial isolates were tested for susceptibility against selected third-generation cephalosporins (ceftriaxone and ceftazidime). Out of 248 bacterial isolates, 140 (56.5%) were found to be resistant to ceftriaxone. But, 37 (14.9%) and 71 (28.6%) of the isolates remain intermediate and susceptible to ceftriaxone, respectively. On the other hand, 149 (60.1%) of the total bacterial isolates were found to be resistant, 53 (21.4%) were intermediate, and only 46 (18.5%) were susceptible to ceftazidime (Figure 1).

As shown in Tables 3 and 4, the rate of bacterial isolates resistant to ceftriaxone and ceftazidime was 56.5% and 60.1%, respectively. Majority of the urinary tract iso- lates were found to be resistant to the action of third- generation cephalosporins (ceftriaxone or ceftazidime). OutFIGURE 1: Resistance profile of clinical isolates to ceftriaxone and ceftazidime of 63 *Escherichia coli* isolates, 46 (73%) were resistant to ceftriaxone which is very high. Moreover, about 41 (65%) of them were resistant to ceftazidime. *Citrobacter freundii,* which is another urinary pathogen, showed a resistance of 36.7% (11/30) to ceftriaxone and 43.3% (13/30) to cef- tazidime.

In this study, most of the Enterobacteriaceae (*Citrobacter koseri*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Enterobacter aerogenes,* and *Proteus* species) isolates were resistant to cef- triaxone or ceftazidime. In addition, *Staphylococcus aureus*, which accounted 19% of total bacterial isolates, showed 23.4% (11/47) and 34% (16/47) resistance to ceftriaxone and ceftazidime, respectively. Similarly, *Klebsiella pneumoniae* showed 46.1% (12/26) resistance to ceftriaxone. More than 90% (10/11) of *Enterobacter aerogenes* were resistant to cef- tazidime and none of the *Proteus* species were susceptible to the action of ceftriaxone or ceftazidime.

The multidrug resistance pattern showed that among the bacterial strains found to be resistant to ceftria- zone and ceftazidime about 109 (44%) and 108 (43.5%) were resistant to two, three, or four drugs, respectively. *Escherichia coli, Staphylococcus aureus, Enterobacter* species, and *Citrobacter* species showed resistance to two, three,

TABLE 2: Sociodemographic characteristics association with resistance pattern of clinical isolates.

Characteristics		Ceftazidime			Ceftriaxone		
		R	NR	P-value	R	NR	P-value
Age in years	≤19	23	20	0.07622	23	20	0.06902
	20-64	80	61		73	68	
≥65		46	18		44	20	
Sex	Female	55	39	0.69326	53	41	0.98641
Male		94	60		87	67	
Specimen Type	Sputum	29	22	0.08527	26	25	0.01426
Urine		41	15		41	15	
	Wound Swab	50	45		45	50	
Stool		29	17		28	18	
Hospital Stay	≤1 Days	35	27	0.29227	30	32	0.35481
	2-3 Days	72	40		66	46	
	4-6 Days	19	20		21	18	
	≥7 Days	23	12		23	12	

TABLE 3: Resistance pattern of the different clinical isolates to ceftriaxone.

Clinical isolates	Resistance pattern			
	Resistant	Intermediate	Susceptible	Total
Citrobacter species	27(52.9%)	13(25.5%)	11(21.6%)	**51**
E. coli	46 (73.0%)	3 (4.8%)	14 (22.2%)	**61**
Enterobacter species	31 (70.4)	5(11.4%)	8(18.2%)	**44**
K. pneumonia	12 (46.2%)	4 (15.4%)	10 (38.4%)	**26**
K. oxytoca	5 (83.3%)	0	1 (16.7%)	**6**
S. aureus	11 (4.4%)	10(4.0%)	26(10.5%)	**47**
Proteus species	4 (80%)	1 (20%)	0	**5**
Y. enterocolitica	4 (66.6%)	1 (16.7%)	1 (16.7%)	**6**
Total	**140 (56.5)**	**37 (14.9%)**	**1 (16.7%)**	**248 (100)**

or four drugs. On the other hand, *Citrobacter* species and *Proteus* species were resistant to two or three drugs while *Klebsiella Pneumonia* revealed resistance to two drugs.

3. Discussion

The widespread use of brood spectrum antibiotics has led to the emergence of antibiotic resistant strains of bacteria. High rates of resistance have been primarily observed in bacteria that cause common health problems. In the present study more than half of the isolated bacteria strains were resistant to either ceftriaxone or ceftazidime drugs which is in agreement with 2014 WHO reports [1].

The drug resistance pattern differences among isolates based on various characteristics were evaluated (Table 2). In view of that, there were no significant differences observed except for the specimen types from which the strains were isolated. Most of the urinary tract isolates were found to be resistant to the action of third-generation cephalosporins (ceftriaxone or ceftazidime). The majority of these isolates were *Escherichia coli* which is a gram-negative bac- terium. This uropathogen is the major extended spectrum beta-lactamase (ESBL) producer, severely limiting the ther- apeutic management in cases of urinary tract infections [23]. Hence, isolates of these strains have relatively high potentials of developing resistance [12].

Moreover, most of *Escherichia coli* strains isolated from the whole specimen were found to be resistant to the action of ceftriaxone and ceftazidime in the present study. It was also revealed that the proportion of resistance to third-generation cephalosporins increased significantly for *Escherichia coli* infections since 2004 [24]. Similarly, other research finding reported that *Escherichia coli* exhibited the highest resistance to ceftazidime and ceftriaxone [25, 26]. However, the study in University of Gondar Hospital strains have a β-lactam ring provided with a Zwitterionic structure that protects these antibiotics from hydrolysis by β-lactamases [32]. On the contrary, the study conducted in Oman stated that most of the isolated strains were sus- ceptible towards third-generation cephalosporin-ceftriaxone [33].

Staphylococcus aureus strains were found to be more susceptible than other bacteria strains to ceftriaxone and ceftazidime which is inconsistent with previous study in which most of the strains were resistant [34]. However, it is in line with other studies conducted in different areas which reported the susceptibility of the strains towards the third- generation cephalosporins [33, 35, 36]. On the other hand, in the study carried out in Dessie Hospital, Ethiopia, the resistance pattern for clinical isolate against ceftriazone was about 43.5% which is more than the present study. These findings indicate that the resistance rate of *Staphylococcus aureus* varies from area to area or/and period to period even within the same country.

Most of the Enterobacteriaceae (*Citrobacter koseri, Enter- obacter cloacae, Klebsiella oxytoca*, *Enterobacter aerogenes,* and *Proteus* species) tested isolates were resistant to ceftriax- one or ceftazidime. Similarly, *in vitro* antimicrobial study in Senegal revealed that most of the isolated Enterobacteriaceae strains were resistant to third-generation cephalosporins [36]. On the other hand, it was reported that *Enterobacter* species were relatively more resistant to ceftriaxone than ceftazidime [37]. Similar resistance pattern with present study was reported for *Enterobacter cloacae* against ceftriazone [38].

Multidrug resistance pattern of isolated strains, which were found to be resistant to either of ceftriaxone and ceftazidime, was also evaluated. The majority of *Escherichia coli* and *Staphylococcus aureus* strains exhibited resistance against two, three, or four antimicrobials. About half of *Escherichia coli* strains resistant to third-generation cephalosporins were also resistant to clinically used drugs such as amikacin, sulfamethoxazole-trimethoprim, piperacillin, and ciprofloxacin. This could be due to the high rate of adaptive mutation. Resistant organisms transfer their resistant genes either to their offspring by replication (vertical gene transfer) or by conjugation where the plasmids

4. Conclusion

Microbial resistance to third-generation cephalosporin drugs have been increasing significantly as the finding of the present study indicated. Moreover, those strains which devel- oped resistance to third-generation cephalosporins were also resistant to multiple drugs which could make treatment of infectious disease triggered by these microbial strains become challenging (Table 5). Therefore, the right medications should be selected based on susceptibility data of causative agents towards the drugs for the treatment of right disease agents.

Data Availability

Complete organized and compiled research data were included in this paper and a complete dataset will be available from the corresponding author on request.

Ethical Approval

This research protocol was reviewed and approved by Insti- tutional Review Board (IRB) of College of Health Sciences of Jimma University by the letter written in Reference no. HRPGC/345/2016 by the Ethical Review Committee.

TABLE 5: Multidrug resistance pattern of microbial strains.

Clinical Isolates	Multi-drug resistance pattern			
	Resistance	Number of isolates	Resistance	Number of isolates
Escherichia coli(n=63)	CTR only	46	CAZ only	41
	CTR, SXT	42	CAZ+SXT	36
	CTR,SXT,AUG	21	CAZ+SXT+AUG	20
	CTR,SXT,AUG,CPR	20	CAZ,SXT,AUG,CPR	19
Klebsiella Pneumonia (n=26)	CTR only	12	CAZ only	19
	CTR,CPR	3	CAZ,CPR	3
	CTR,CPR,AMK	0	CAZ,CPR,AMK	0
Staphylococcus aureus (n=47)	CTR only	11	CAZ only	16
	CTR,CPR	6	CAZ,CPR	7
	CTR,CPR,AUG	2	CAZ,CPR,AUG	2
Citrobacter species (n=51)	CTR only	27	CAZ only	29
	CTR,PIP	25	CAZ,PIP	27
	CTR,PIP,CPR	9	CAZ,PIP,CPR	9
	CTR,PIP,CPR,AMK	1	CAZ,PIP,CPR,AMK	0
Enterobacter species (n=44)	CTR only	32	CAZ only	36
	CTR,PIP	30	CAZ,PIP	33
	CTR,PIP,CPR	13	CAZ,PIP,CPR	14
	CTR,PIP,CPR,AMK	1	CAZ,PIP,CPR,AMK	1
Proteus species (n=5)	CTR only	4	CAZ only	3
	CTR+PIP	3	CAZ+PIP	2
	CTR,PIP,CPR	1	CAZ+PIP+CPR	1
	CTR,PIP,CPR,AMK	0	CAZ,PIP,CPR,AMK	0

CTR= ceftriaxone, SXT = sulfamethoxazole-trimethoprim, AMK= amikacin,, PIP= piperacillin, CAZ= ceftazidime,, AUG= Amox-clavulanic acid, and CPR= ciprofloxacin.

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