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Antimicrobial Resistance Profile of Different Clinical Isolates against Third-Generation Cephalosporins

[1]DR B V RAMANA,[2]S.K.RUBINA,[3]DR M SANTOSH RAJA,
[4]A HEMANTH,[5]S.AFREEN JEHA

Department Of Pharmaceutics

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Abstract
death because they increase the likelihood of harmful treatment. Antimicrobial resistance statistics therefore aid in determining optimal patient care. Consequently, the purpose of this research was to examine the antimicrobial resistance pattern of third-generation cephalosporin medications at Jimma University Specialized Teaching Hospital. Methods. From April to August 2016, researchers at Jimma University Specialized Hospital (JUSH) performed a prospective cross-sectional study in a hospital setting. Patients in hospitals had their urine, sputum, wound swabs, and feces collected for clinical purposes. Following this, the usual microbiological procedures were followed in order to isolate and identify the bacterial species. Using the Kirby-Bauer disc diffusion technique, we tested the susceptibility of different microorganisms to antimicrobials. Final product. Out of a total of 248 bacterial isolates, 154 (62.1%) were found in male patients and 94 (37.0%) were found in female patients. Specimens were most often found to include Escherichia coli (25.4% of the total) and Staphylococcus aureus (19.0%). Approximately 56.5% of the total bacterial isolates (140) and 60.1% (149) were determined to be ceftriaxone and ceftazidime resistant, respectively. Most of the Escherichia coli isolates were resistant to ceftriaxone (73%) and ceftazidime (65%), with 41 (65%) of the isolates showing resistance to both drugs. Of all the germs tested, 19% were Staphylococcus aureus, and of those, 23.4 percent were resistant to ceftriaxone and 33.4 percent to ceftazidime, respectively. Out of the bacterial strains that showed resistance to ceftriazone and ceftazidime, about 109 (44% of the total) were also resistant to two, three, or four additional medicines. In summary. More and more bacterial strains are showing resistance to third-generation cephalosporins, such as ceftriaxone and ceftazidime. This indicates that the problem is becoming worse. Treatment may fail because some bacteria have shown multidrug resistance, especially to medicines used in clinical settings. Thus, antimicrobial susceptibility test results should inform the selection of suitable drugs for clinical usage.

Introduction

One of the greatest threats to public health throughout the world in the modern era is the rise of bacteria and other microbes that are resistant to antibiotics [1]. There has been a dramatic increase in the number of drug-resistant microbial strains, the number of regions hit hard by this problem, and the degree to which resistance manifests in individual organisms [2]. More worryingly, the proportion of organisms displaying AMR, particularly resistance to several antibiotics, is steadily rising [3]. So, bacteria and viruses that were hitherto believed to be sensitive to antibiotics are now showing signs of becoming resistant to them in unprecedented numbers [4].

An rise in morbidity and death is caused by antibiotic-resistant bacteria because they raise the risk of inappropriate treatment [5, 6]. The therapy might be delayed or hindered due to this resistance, which could cause problems or even death [7, 8]. In addition, patients may need more intrusive therapies, such intravenous injections, to be administered in hospitals, and they may have to take more costly and different antibiotics, which may have more severe side effects [6, 9].
Therapy becomes more precarious, expensive, and sometimes ineffective due to multi-resistant organisms. Infections that are resistant to many drugs may be fatal for people.

2 because, particularly in the poor world, no medication has ever worked [10]. One example is the danger that multidrug-resistant (MDR) enteric disease pathogens pose to public health in underdeveloped nations [3]. Multiple drug-resistant strains of many bacteria have been identified in various parts of the world, including Mycobacterium tuberculosis, Enterococcus faecium, Enterobacter cloacae, Klebsiella pneumoniae, Staphylococcus aureus, Acinetobacter baumanii, and Pseudomonas aeruginosa [11].
Empirical treatment, which relies on the clinician's prior clinical knowledge, has historically been effective in treating many infections [12, 13]. Nevertheless, with the emergence of resistance to almost every antimicrobial drug now licensed for use in human and veterinary clinical treatment, this approach is rapidly becoming the norm rather than the exception. This, on top of the wide range of antimicrobial agents on the market today, makes choosing the right one a formidable challenge. This condition emphasizes the significance of the diagnostic laboratory in clinical practice and has made doctors rely increasingly on results from in vitro antimicrobial susceptibility testing [14].
Data on antimicrobial resistance (AMR) among local pathogens aids in determining the optimal course of therapy for specific patients [15, 16]. Nevertheless, there is no consistent data on resistance trends at many healthcare institutions, and the percentage of resistant bacteria might differ from one region to another [17, 18]. Results from antimicrobial resistance (AMR) surveillance networks demonstrate that data, when accessible, can serve several purposes, such as guiding treatment decisions, illuminating trends in AMR, shaping public health policy, pinpointing intervention priority areas, and tracking the effectiveness of interventions to curb resistance [1]. But there is a lack of information on the antibiotic resistance profile, which is particularly problematic in poor nations like Ethiopia. As a result, the current investigation screens the antimicrobial resistance profile of third-generation cephalosporin medications used to treat infectious infections at Jimma University Specialized Teaching Hospital.

2.Content and Procedures

2.1.Study Design and Specimen Collection. The Jimma University Specialized Hospital (JUSH) was the site of a cross-sectional research that ran from April 2016 to August 2016. Patients from all across the nation seek treatment at this hospital, and it's chosen for the variety of medical specialties it offers. Certified nurses took swabs from wounds, urine, sputum, and stool samples from patients in the hospital.

In Section 2.2, We Discuss Bacteria Identification. We used established microbiological techniques to gather all of the clinical samples in order to identify and isolate harmful germs. Next, the specimens were placed onto several types of agar and incubated aerobically at 37◦ C for 24 hours, depending on their source. These agars include MacConkey, Blood, Mannitol Salt, Xylose lysine deoxycholate, Chocolate, and Thayer-Martin.
Typically yellow to golden in hue, clustered gram-positive cocci that are catalase and coat protein positive

To distinguish Staphylococcus aureus from other gram-positive cocci, the bacteria were cultured on blood agar and then fermented with mannitol on MSA. Yersinia enterocolitica, gram-negative bacilli, coliforms, and Proteus spp. were all identified using the standard microbiological algorithms described in the reference material, which included gram's stain (for gram-negative bipolarly stained bacilli), colonial growth characteristics, and the appearance on enriched and selective media [19]. The relevant and clinically significant clinical isolates were identified using biochemical tests such as lysine decarboxylation (LDC), indole and citrate utilization (MIS), methyl red (MR), Voges-Proskauer (VP), and pyrrolidonyl aminopeptidase (PYR) [19, 20]. Therefore, the obtained clinical samples included Yersinia enterocolitica, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus species, Citrobacter freundii, Citrobacter Koseri, Enterobacter cloacae, Klebsiella oxytoca, and Enterobacter aerogenes.

The 2.3. Antimicrobial Susceptibility Test. Using S. aureus ATCC 25923 and other quality control strains, antimicrobial susceptibility testing was conducted using disk diffusion technique in accordance with the Kirby-Bauer method [21]. Consequently, at least three or five distinct colonies with matching morphologies were chosen from an agar plate culture, moved into Muller Hinton broth, and kept at 37◦ C for a day. To get a turbidity visually similar to the 0.5 McFarland standards, the suspension's turbidity was modified using sterile saline. The newly produced Mueller Hinton agar plate was then streaked with the swab. The antimicrobial disks were placed on top of the plates no later than fifteen minutes after inoculation. The next step was to incubate the plates at 37◦ C for a full day. Using resistance data interpreted in accordance with the Clinical and Laboratory Standards Institute [22], the results of measuring the zone of inhibition were classified as sensitive, resistant, or intermediate. The third-generation cephalosporins ceftriaxone (30 g) and ceftazidime (CAZ) (30 g) were the antibacterial agents that were examined. Additionally, multidrug-resistant (MDR) profiles for cephalosporin-resistant bacteria were established using various classes of antimicrobials, including ciprofloxacin (5 g), sulfamethoxazole-trimethoprim (25 g), amikacin (AMK) (30 g), piperacillin (PIP) (100 g), Amox-clavulanic acid (AUG), and ciprofloxacin (CPR) (5 g). Discs containing antibiotics were exclusively produced by Abtek Biologicals Ltd. of Liverpool, UK, L9 7AR.

We control the quality in section 2.4. By incorporating quality control methods into every step of the laboratory activity, the study's results could be relied upon. Prior to usage, the typical expiration dates of staining reagents, culture medium, and antibiotic discs were verified. Following their preparation and sterilization by autoclaving at 121◦ C for 15 minutes, all culture plates and antibiotic discs were placed in the refrigerator at the appropriate temperature. The normative

TABLE 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.

l.l. 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 statistically significant differences and the results were presented using tables and figure.

2. Results

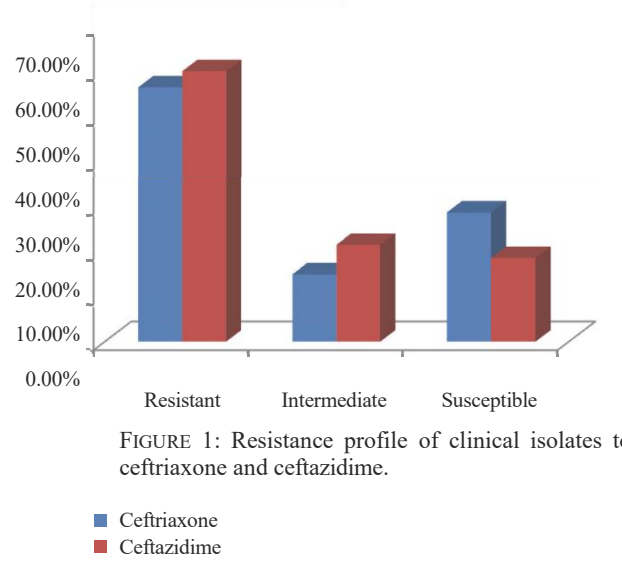

FIGURE 1: Resistance profile of clinical isolates to ceftriaxone and ceftazidime.

infections affecting the upper respiratory tract. Out of 154 (62.1%) male and 94 (33.9%) female individuals, 248 (64% of the total) bacterial isolates were collected. The most common bacteria found in the samples taken from the participants in this investigation were Staphylococcus aureus (19.0%) and Escherichia coli (25.4%). According to Table 1, the remaining bacterial isolates consist of the following: Citrobacter freundii (12.1%), Citrobacter koseri (8.5%), Enterobacter cloacae (14.0%), Klebsiella oxytoca (2.4%), Klebsiella pneumoniae (10.5%), Enterobacter aerogenes (4.8%), Proteus species (2.0%), and Yersinia enterocolitica (2.4%).

To determine which third-generation cephalosporins (ceftriaxone and ceftazidime) were most effective against the bacteria, we examined each isolate. One hundred forty-four (56.5% of the total) bacterial isolates tested negative for ceftriaxone. On the other hand, 71 (28.6% of the total) and 37 (14.9%) of the isolates are still sensitive to ceftriaxone. Figure 1 shows that out of all the bacterial isolates, 110 were resistant to ceftazidime, 53 were intermediate, and just 46 were susceptible.

Tables 3 and 4 reveal that 56.5% of the bacterial isolates were resistant to ceftriaxone, whereas 60.1% were resistant to ceftazidime. It was discovered that most isolates from the urinary tract were resistant to third-generation cephalosporins, such as ceftriaxone or ceftazidime. Out

Out of 63 Escherichia coli isolates, a staggering 73% (46 out of 63) showed resistance to ceftriaxone. Plus, ceftazidime was ineffective against around 41 (or 65%) of them. Another urinary tract infection, Citrobacter freundii, exhibited resistance to ceftriaxone at a rate of 36.7% and cef-tazidime at a rate of 43.3%.

All of the Enterobacteriaceae species tested here were resistant to either ceftriaxone or ceftazidime, including Citrobacter koseri, Enterobacter cloacae, Klebsiella oxytoca, Enterobacter aerogenes, and Proteus species. Furthermore, among all bacterial isolates, Staphylococcus aureus constituted 19%. Of these, 23.4% (11/47) were resistant to ceftriaxone, while 34% (16/47) were resistant to ceftazidime. Similarly, ceftriaxone resistance was observed in 46.1% (12/26) of Klebsiella pneumoniae. Ceftriaxone and ceftazidime were ineffective against any species of Proteus, and more than 90% of Enterobacter aerogenes (10/11) were resistant to them.

Among the bacterial strains that were shown to be resistant to ceftria-zone and ceftazidime, about 109 (44%) and 108 (43.5%), respectively, exhibited multidrug resistance. This means that these bacteria were resistant to two, three, or four medicines. Some species of Enterobacter, Staphylococcus aureus, Citrobacter, and Escherichia coli were resistant to two, three,

The correlation between sociodemographic variables and the resistance pattern of clinical isolates is shown in Table 2.

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 bacterium. This uropathogen is the major extended spectrum beta-lactamase (ESBL) producer, severely limiting the therapeutic 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, Ethiopia, showed that the percentage of resistance strains observed against ceftazidime was high but relatively less to ceftriaxone [27].

The majority of *Klebsiella pneumoniae* strains were more resistant to ceftazidime compared to ceftriazone in this study. However, it is dissimilar with other studies which showed that the isolates exhibited similar resistance pattern to both ceftazidime and ceftriaxone [28, 29]. It was also reported that *Klebsiella pneumoniae* strain isolated from patients with community acquired pneumonia was resistant to third-generation cephalosporins [30, 31]. This is because these

4: Resistance pattern of the different clinical isolates to ceftazidime.

Clinical isolates	Resistance pattern			
	Resistant	Intermediate	Susceptible	Total
E. coli	41 (65.1%)	10 (15.9%)	12 (19.0%)	**63**
Citrobacter species	29 (56.9%)	8 (15.7%)	14 (27.4%)	**51**
S. aureus	16 (34.0%)	20 (42.6%)	11 (23.4%)	**47**
Enterobacter species	35 (79.6%)	6 (13.6%)	3(6.8%)	**44**
K. pneumonia	19 (73.1%)	5 (19.2%)	2 (7.7%)	**26**
K. oxytoca	4 (80%)	2 (20%)	0	**6**
Y. enterocolitica	2 (33.3%)	0	4 (66.7%)	**6**
Proteus species	3 (60.0%)	2 (40.0%)	0	**5**
Total	**149 (60.1%)**	**53 (21.4%)**	**46 (18.5%)**	**248 (100)**

These antibiotics are protected against hydrolysis by -lactamases because some strains possess a -lactam ring that is furnished with a Zwitterionic structure [32]. The majority of the isolated strains were susceptible to the third-generation cephalosporin, ceftriaxone, according to the Oman research [33].

In contrast to earlier research that indicated that the majority of Staphylococcus aureus strains were resistant to ceftriaxone and ceftazidime, our current investigation demonstrated that Staphylococcus aureus strains were more responsive to these antibiotics [34]. Nonetheless, the results are consistent with previous research that found the strains to be susceptible to third-generation cephalosporins [33, 35, 36]. Contrarily, a higher percentage of clinical isolates showed resistance to ceftriazone in a research conducted at Dessie Hospital in Ethiopia (43.5%). These results show that even within a single nation, the rate of Staphylococcus aureus resistance differs between regions and time periods.

Enterobacter aerogenes and ceftriaxone/ceftazidime resistance was seen in the majority of the Enterobacteriaceae (Citrobacter koseri, Enterobacter cloacae, Klebsiella oxytoca, and Proteus species) isolates investigated. Similarly, Senegalese in vitro antimicrobial research showed that third-generation cephalosporins were ineffective against the majority of the Enterobacteriaceae strains tested [36]. Contrarily, research has shown that ceftriaxone is more effective against Enterobacter species than ceftazidime [37]. There was a similar trend of resistance to ceftriazone in a previous investigation including Enterobacter cloacae [38].

Isolated isolates that showed resistance to both ceftriaxone and ceftazidime were also tested for multidrug resistance. The vast majority of Staphylococcus aureus and Escherichia coli strains were resistant to at least two antibiotics. A significant portion of the Escherichia coli bacteria that exhibited resistance to third-generation cephalosporins were also resistant to other commonly prescribed medications, including amikacin, ciprofloxacin, sulfamethoxazole-trimethoprim, and piperacillin. The rapid pace of adaptive mutation may be to blame for this. One way that resistant organisms pass their genes on to new generations is by conjugation, which involves plasmids, or through replication, which is also known as vertical gene transfer.

4. Conclusion

The current study's findings demonstrated a dramatic rise in microbial resistance to third-generation cephalosporin medications. Treatment of infectious diseases caused by these bacteria strains may become more difficult as they developed resistance to third-generation cephalosporins and several other medications (Table 5). Accordingly, in order to treat the correct illness agents, it is necessary to use data on the sensitivity of the agents to the pharmaceuticals to choose the appropriate treatments.

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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[38] Citation: "Antimicrobial susceptibility pattern of bacteria isolated from patients with urinary tract infection" (I. U. Khan, I. A. Mirza, A. Ikram et al., 2014, vol. 24, no. 11, pp. 840-844, Journal of the College of Physicians and Surgeons Pakistan).

In their study published in BMC Research Notes, P. Baral, S. Neupane, B. P. Marasini, K. R. Ghimire, B. Lekhak, and B. Shrestha found that

bacterial uropathogens from Kathmandu, Nepal, had a high incidence of multidrug resistance.