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Original Research Article

Multi Drug Resistance, Extensive Drug Resistance, and Pan Drug Resistance Enterobacterales from Clinical Samples in Usmanu Danfodiyo University Teaching Hospital Sokoto

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Abstract: Antimicrobial resistance (AMR) is now recognized as one of the most serious global threats to human health in the 21st century. This study is set out to investigate the resistance pattern of Enterobacterales from clinical isolates in Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. Three hundred and twenty isolates of Enterobacterales were isolated from different clinical samples and identified using the Analytical Profile Index 20E. Antimicrobial susceptibility testing was performed using the modified Kirby-Bauer disk diffusion method, and the antibiotics tested include Augmentin, Ciprofloxacin, Ceftriaxone, Ceftazidime, Cefepime, Penicillin G, Amikacin, Meropenem, Cotrimoxazole and Gentamicin. Classification into multi drug resistance (MDR), Extensive drug resistance (XDR) and Pan drug resistance (PDR) was done using the international expert proposal for interim standard definitions for acquired resistance. Data was analyzed using statistical package for social sciences v23. *Escherichia coli* 165 (51.6%) was the most often isolated bacterium, followed in that other by *Klebsiella pneumoniae* 93 (29.1%). The highest level of resistance was observed in Co-trimoxazole 278 (86.9%), while the least was observed in Meropenem 49 (15.3%). Amikacin 92 (28.7%), Augmentin 178 (55.6%), Ceftriaxone 164 (51.4%), Ceftazidime 187 (58.4%), Gentamicin 192 (60.0%), Cefepime 113 (35.3%), Ciprofloxacin 120 (43.8%), and Penicillin G 278 (86.9%). Multi drug, Extensive drug and Pan drug resistance accounts for 80.3%, 11.9% and 4.0% respectively. The prevalence of the quinolone resistance gene was found to be 5.3%. This study addressed some key knowledge gaps as pertains to antibiotic sensitivity and resistance patterns in our region, making a significant contribution towards filling the global resistance map.

Keywords: Enterobacterales, Multi Drug Resistance, Extensive Drug Resistance and Pan Drug Resistance.

INTRODUCTION

Gram-negative bacilli belonging to the order Enterobacterales and family Enterobacteriaceae are the most frequently encountered bacterial isolates recovered from clinical specimens. Widely dispersed in nature, these organisms are found in soil and water, on plants, and, as the family name indicates, within the intestinal tracts of humans and animals [1]. Thus, members of the Enterobacteriaceae may be incriminated in virtually any type of infectious disease and recovered from any specimen received in the laboratory [2]. Immunocompromised or debilitated patients are highly susceptible to hospital-acquired infections, either after colonization with environmental strains or following invasive procedures, such as catheterization, bronchoscopy, colposcopy, or surgical biopsies, in which mucous membranes are traumatized or transected.

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Characteristic colonial morphology of an organism growing on a solid medium may provide a second clue [3]. Antimicrobial resistance (AMR) is now recognized as one of the most serious global threats to human health in the 21st century. There is now evidence of political traction, with endorsements of statements by the World Health Organization (WHO) and United States (US) Centers for Disease Control and Prevention (CDC) describing a global crisis and an impending catastrophe of a return to the pre-antibiotic era [4]. These serious concerns have been catalyzed by the rapid increase in carbapenemase-producing Enterobacterales. Drug resistance patterns can be used to categorize micro-organisms into several phenotypes. A multi-drug resistant (MDR) bacteria is said to occur when the organism is resistance to a minimum of three antimicrobial agents from different class, such as aminoglycosides, carbapenems, cephalosporins and/or fluoroquinolones. The strains known as extensively drug-resistant (XDR) are only susceptible to one or two antimicrobial agent from different class. All antimicrobial agents cannot affect strains that are pan-drug resistant (PDR) [5].

World Health Organisation has declared 12 different families of bacteria as priority pathogens [6]. It is important to determine the global prevalence of these highly resistant organisms in order to implement control and prevention strategies. Effective antibiotics are seriously needed to improve the already compromised modern clinical medicine particularly infectious and surgical procedures [7]. Despite growing information on the global spread and distribution of resistant pathogens, very few studies have looked into the prevalence of this pathogens in African countries [8].

This study is set out to investigate the resistant pattern of Enterobacterales from clinical isolates in Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria.

MATERIAL AND METHODS

This is a hospital based descriptive cross sectional study that was conducted in the department of Medical Microbiology Usmanu Danfodiyo University Teaching Hospital Sokoto (UDUTH) is located in Sokoto State, the north-western Nigeria [9]. The study population were adult patients with clinical diagnosis of infectious disease. This study was carried out within nine months (March to November 2023). The sample size required for this study was obtained using the formula according to Fischer formula [10].

Sample Processing

About 320 Enterobacterales were collected from different clinical sample depending on the clinical diagnosis. Choice of medium was determined by the type of sample. Cystein lactose electrolyte deficient (CLED) agar and blood agar was used for urine, MacConkey and chocolate agar for CSF and blood, Salmonella Shigella Agar (SSA), and Xylose Lysine Deoxycholate (XLD) agar for stool, MacConkey and blood agar for sputum, aspirate and swab. After 16-18 hours of aerobic incubation at 36-37 °C, colonial appearance and characteristics of isolates on the agar was noted and subjected to Gram staining reaction according to standard methods [11]. All suspected isolates of Enterobacterales were confirmed by the Analytical Profile Index (Biomerieux) 20E (API 20E) according to the manufacturer's instructions [12].

Antimicrobial Susceptibility Testing

Modified Kirby-Bauer disc diffusion method was used according to the Clinical Laboratory Standard Institute (CLSI 2022) guidelines [13]. No more than six antibiotics was tested in a 100mm plate. The disc was placed no more than 24 mm apart from center to center. Each disc was gently pressed down to ensure complete contact with the agar surface and do not fall when the plate is inverted during incubation. The plate was placed in an incubator at 35 °C$\pm$ 2^0C within 30 minutes of preparation for 16-20hrs. After overnight incubation, the diameter of the zone of complete inhibition (as a judged by the unaided eye) was measured including the diameter of the disc with a ruler or caliper on the under-surface of the plate without opening the lid and recorded in mm. The results were interpreted sensitive, intermediate and resistance according to the CLSI 2022 [13].

Molecular Detection of Quinolone Resistance Gene

Ciprofloxacin was used as a marker for quinolone and the quinolone resistance (qnr) gene was searched for among the ciprofloxacin resistant isolate.

The Run Mei Genomic DNA Extraction Kit was used to extract total nucleic acids from the resistant bacterial isolates. Nucleotide sequences of QnrB (F: GGMATHGAAATTCGCCACTG R: TTTGCYGYYCGCCAGTCGAA); Amplicon size = 264 bp; QnrB gene.

The PCR condition was initial denaturation at 95^0C for 15 min, then 30 cycles of 95^0C for 1 min, 55^0C for 1 min, and 72^0C for 5 minutes, and one cycle of final elongation at 72^0C. The PCR products was analyzed after electrophoresis in 1.0% agarose gel to detect specific amplified products by comparing with standard molecular weight markers. The amplified products of the study samples were visualized by a trans-illuminator. And the gel was photographed by a digital camera and transferred data to the computer for further documentation [14].

RESULTS

The participants' ages ranged from 18 to 72 years old. The mean age was 36.38 years, with a standard deviation of ±13.6%. The highest age range was seen in the participants with age range between 28–37 years, 97 (30.4%), and the least were 68–77 years, 12(3.6%). There were 122 (38.1%) female participants and 198(61.9%) male participants in total. The most common provisional diagnosis 84(26.3%) was urinary tract infections. With a frequency of 108(33.9%), urine was the sample that produced a positive result the most often, followed by swabs with 107(35.5%) and blood with 7(2.2%). Sputum, aspirate, stool, bodily fluid and tissue culture are among the additional samples; their frequencies are 15(4.7%), 18(5.6%), 24(7.5%), and 1(0.3%).

Escherichia coli 165 (51.6%) was the most often isolated bacterium, followed in that other by *Klebsiella pneumoniae* 93 (29.1%). The highest level of resistance was observed in Co-trimoxazole 278 (86.9%), while the least was observed in Meropenem 49 (15.3%). Amikacin 92 (28.7%), Augmentin 178 (55.6%), Ceftriaxone 164 (51.4%), Ceftazidime 187 (58.4%), Gentamicin 192 (60.0%), Cefepime 113 (35.3%), Ciprofloxacin 120 (43.8%), and Penicillin G 278 (86.9%). MDR, XDR and PDR accounts for 80.3%, 11.9% and 4.0% respectively. The prevalence of the qnr gene was found to be 5.3%.

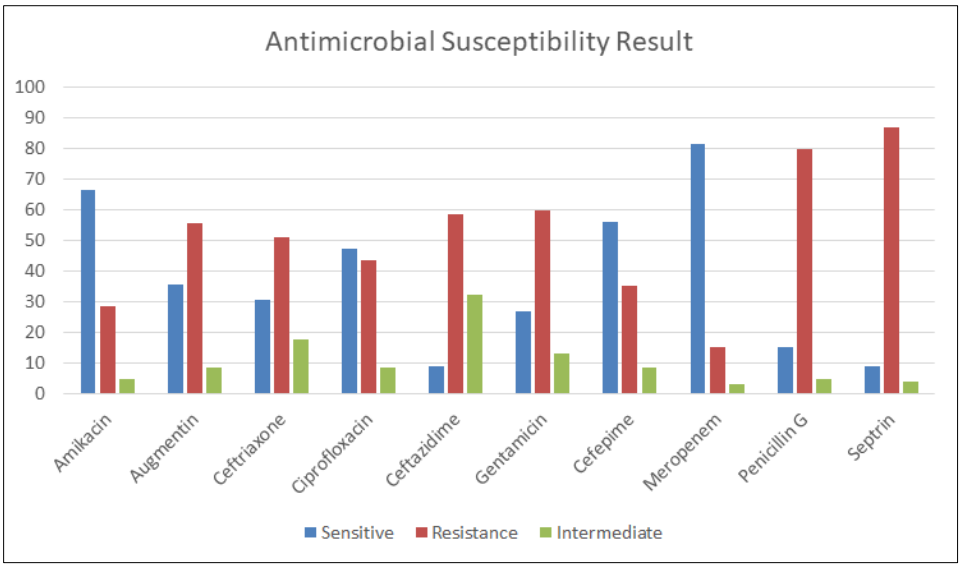

Figure 1: Antibiotic Susceptibility of the Enterobacterales

Table 1: Prevalence of Multi Drug Resistance, Extensively Drug Resistance and Pandrug Resistance

Enterobacterales	**Non-MDR**	**MDR**	**XDR**	**PDR**
Citrobacter spp	0.0	80.0	20.0	0.0
Enterobacter spp	0.0	28.5	57.1	14.2
Erwinia spp	0.0	0.0	100.0	0.0
Escherichia coli	2.3	84.1	12.3	1.4
Klebsiella spp	7.7	84.6	7.7	0.0
Pantoea spp	0.0	100.0	0.0	0.0
Proteus spp	6.1	81.8	12.1	0.0
Salmonella spp	37.5	50.0	12.5	0.0
Serratia spp	0.0	100.0	0.0	0.0
Shigella spp	0.0	0.0	100.0	0.0
Resistance pattern	**Frequency**	**Percentage (%)**		
Non-MDR	12	3.8		
MDR	257	80.3		
XDR	38	11.9		
PDR	13	4.0		
MDR: Multidrug resistance XDR: Extensive drug resistance PDR: Pan drug resistance				

Figure 2: Gel electrophoresis showing the qnr gene

DISCUSSION

The resistance patterns shown by these organisms is quite worrisome and call for a need to strengthen antimicrobial stewardship in the facility and the state at large. Meropenem is shown to be the most sensitive antibiotic, at 81.3%, followed by Amikacin at 66.3%. The two antibiotics are supposed to be reserved antibiotics in the hospital and fall under the Watch category in the WHO-aware classification of antibiotics [6]. This disagrees with the study by Tobin and colleagues from Edo state that reported a 100% sensitivity to meropenem [15]. The study was carried out on outpatients who are probably harboring community-acquired pathogens, which often exhibit a superior sensitivity pattern in comparison to those acquired in healthcare facilities. A high resistance of 65.3% was reported by Medugu *et al.,* in their study [16]. The study is a multi-center study targeting multidrug-resistant organisms. Furthermore, the highest level of resistance was seen in Septrin, at 86.9%. This is slightly lower than what was reported in other studies. The high level of resistance to cephalosporin, penicillin, and quinolones shown by the isolates is in tandem with research from other parts of the country and Africa [17]. In other regions of the world, where there are efficient mechanisms in place to curb it, the situation is different [18]. Antimicrobial resistance (AMR) in poor nations has many fundamental causes, including patient behavior toward antibiotic usage, health care providers' behaviors, and antimicrobial supply networks among the general population. Unsuitable prescribing practices, poor patient education, a lack of diagnostic resources, the selling of antibiotics without authorization, the absence of effective drug regulation systems, and the use of antibiotics for purposes other than human consumption, such as animal husbandry, are a few of these variables [19]. Intervention programs in developing countries must address the context and concentrate on the root causes unique to this region of the world, given that these elements may differ in developing from industrialized nations [20].

All isolates from this study exhibited resistance to antibiotics commonly used in hospitals. More than 80% of all isolates were found to be MDR, with XDR and PDR having prevalence of 11.9% and 4.0% respectively. The findings of this study is similar to what was reported from Nigeria by Usman *et al*., In his study 3 (9.4%) are pan-drug resistant strain, 10(31.2%) were extended-drug resistant strain while 19 (59.3%) were multidrug-resistant strain out of the 32(100%) positive *Klebsiella pneumonia* that were examined [21]. Similar findings has also been reported from South Africa, Ghana and other African countries [22- 24]. Globally, AMR have become a major concern with a very high prevalence of MDR, XDR and PDR [25, 26]. An intriguing finding was found by Pal *et al.,* from India, More than 95% of all isolates were found to be multiple drug resistant (MDR) and more than 50% were possible extensively drug-resistant (XDR). None of the isolates were pan drug-resistant (PDR) [27]. Worldwide, the magnitude of the problem and its effect on animal and human and its impacts on economies are poorly

documented. However, estimates record an untenably high burden that will largely be borne by low-income countries such as Nigeria [28].

CONCLUSION

This study addressed some key knowledge gaps as pertains to antibiotic sensitivity and resistance patterns in our region, making a significant contribution towards filling the global resistance map. There was overwhelming resistance noted to commonly used antibiotics such as penicillins and cephalosporins. Rising resistance to potent antibiotics such as carbapenems posed a cause of concern. Collaborative efforts involving clinicians with other key stakeholders are needed to strengthen antimicrobial stewardship efforts, and promote regular surveillance and further research towards combating antimicrobial resistance for the present and future generations. This emphasizes the necessity of putting in place antimicrobial stewardship programs that support the responsible use of antibiotics and motivate medical professionals to only prescribe these drugs when absolutely necessary and in compliance with established protocols.

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