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Antimicrobial Susceptibility Pattern of Clinical Isolates of *Escherichia coli* and *Proteus mirabilis* Using Cefepime in Ogbomoso, Nigeria.

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Abstract

The challenge of Cefepime resistance in developing countries is substantial and likely to grow. Emergence of Cefepime-resistant bacteria has increased and management of this has become a therapeutic challenge. The production of beta lactamases by most bacteria make them to be resistant to beta lactam antibiotics like Cefepime which is common nowadays. Forty clinical isolates of *Escherichia coli* and *Proteus mirabilis* from different clinical sites were analyzed between March and August, 2013 using standard bacteriological methods. The aim of this study was the evaluation of the sensitivity of clinical isolates of *Escherichia coli* and *Proteus mirabilis* to Cefepime. Their sensitivity against Cefepime (30µg) was determined using disk diffusion method. The isolates were divided into three groups: sensitive, intermediate and resistance based on their sensitivity pattern.

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The result showed that *Escherichia coli* had 29.03% sensitivity, 16.13% intermediate and 58.83% resistance while *Proteus mirabilis*had 22.22% sensitivity, 11.11% intermediate and 66.67% resistance. The overall susceptibility pattern of the clinical isolates to Cefepime is 27.50% sensitivity, 15.00% intermediate and 57.57% resistance. There was a great Cefepime- resistance among clinical isolates of *E. coli* and *Proteus mirabilis* analyzed. The resistance pattern of *E. coli* and *Proteus mirabilis* calls for continuous surveillance for Cefepime resistance control.

Keywords:Cefepime; LAUTECH; Ogbomoso; *Escherichia coli;Proteus mirabilis; Sensitivity*

1. Introduction

Enterobacteriaceae are a major cause of infections in hospitalized patients [1]. They are large family of bacteria that causes both nosocomial and community-acquired infections. This family includes many more familiar pathogens, such as *Salmonella* spp. and *Escherichia coli*, as well as *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., and others [2]. Among them, the most frequent are *Escherichia coli* and *Enterobacter* spp [3]. *Proteus mirabilis* strains account for about 10 % of uncomplicated urinary tract infections [4] and they are about the fifth most common cause of nosocomial urinary tract infections and sepsis in patients [5, 6]. Uro- pathogenic *Proteus mirabilis* and *Escherichia coli* strains may manifest resistance to several antimicrobials, including extended spectrum cephalosporins, fluoroquinolones, and aminoglycosides [7,8, 9] . Extended-Spectrum β-Lactamase (ESBL) producing strains of *Enterobacteriaceae* have emerged as a major problem in hospitalized as well as community based patients [10]. These organisms are responsible for a variety of infections like urinary tract infection (UTI), septicemia, hospital acquired pneumonia, intra-abdominal abscess, brain abscess and related infections. ESBLs are primarily produced by the Enterobacteriaceae family, in particular *Escherichia coli* [11] and are a major cause of hospital acquired infections [12]. *Proteus* is widespread in the environment and ranks third as the cause of hospital-acquired infection [13]. *Proteus* species are major cause of diseases acquired outside the hospital, where many of these diseases eventually required hospitalization [14]. *P. mirabilis* has been implicated in bacteremia [15] , neonatal meningoencephalitis [16] , empyema [17] and osteomyelitis [18]. The usefulness of third-generation Cephalosporins may be diminished by the presence of inducible β-lactamases in important nosocomial pathogens [19]. Newer generation of cephalosporins such as cefepime, however, retain activity against the strains of Gram-negative bacilli that produce plasmid-mediated β-lactamases compared to ceftazidime [20].

2. Material and Methods

2.1 Bacterial Isolates

The study includes clinical isolates of *Escherichia coli* and *Proteus mirabilis* obtained by screening samples of blood (for blood culture), urine, aspirate, wound, throat, sputum, etc. Forty isolates of *Escherichia coli* and *Proteus mirabilis* were identified from different clinical specimens between March and August, 2013 using standard bacteriological methods.

Clinical isolates were isolated and obtained from Medical Microbiology and Parasitology Laboratory of the Ladoke Akintola University of Technology Teaching Hospital, Ogbomoso,Oyo State, Nigeria. LAUTECH Teaching Hospital, Ogbomoso provides a tertiary level patient care, serves as referral hospital in Ogbomoso and provides health care facilities to people of different areas.

The clinical isolates were identified based on their morphological behavior on various differential media. All media were prepared according to the manufacturer's specification and sterilized at 121°C for 15 minutes at 15 lb pressure. Further identification was then carried out by standard biochemical test (Bergey's Manual of Determinative Bacteriology Ninth Edition) and by comparing their characteristics with those of known taxa, as described by [21, 22,23].

2.2 Susceptibility Test

The susceptibility test was conducted using the Kirby- Bauer method of Sensitivity determination. Petri – dishes of Mueller Hinton agar were prepared according to the manufacturer's instruction. 0.1ml of *Escherichia coli* and *Proteus mirabilis* equivalent to 0.5 McFarland standard was seeded into each of the Petri-dishes containing Mueller-Hinton agar using sterile swabs. These were allowed to stand for 45 minutes to enable the inoculated organisms to pre-diffusion. The antibiotics discs of Cefepime (30µg ;Oxoid, UK) were aseptically placed on the surfaces of the sensitivity agar plates. These were incubated for 18 – 24 hours at 37^{0} C and the radial zone of inhibitions were taken.

The results were expressed as susceptible, intermediate or resistant according to criteria developed by Clinical Laboratory Standard Institute (CLSI), 2007.

2.3 Statistical Analysis

Susceptibility rates were analyzed using t- test, at significant level of $p \leq 0.05$. All interval estimates are 95% confidence intervals. SPSS program for Windows (version 16.0; SPSS, Inc., Chicago, IL) was used.

3. Results

Forty clinical isolates of *Escherichia coli* and *Proteus mirabilis* were obtained from different clinical sites as shown in Table 1. Out of the forty clinical isolates, 31 (77.50%) were *Escherichia coli*, and 9 (22.50%) were *Proteus mirabilis*. It is clearly shown that *E. coli* is more associated with urine specimens, while *Proteus mirabilis* are more common in wound specimen because the highest number of clinical isolates of *E. coli* was found in urine specimen while that of *Proteus mirabilis* was found in wound specimen.

Escherichia coli had 29.03% sensitivity, 16.13% intermediate and 54.83% resistance while *Proteus mirabilis* had 22.22% sensitivity, 11.11% intermediate and 66.67% resistance as shown in table 2. The grand susceptibility pattern of the organisms to cefepime is 27.50% sensitivity, 15.00% intermediate and 57.50% resistance. The highest resistance to cefepime was found in *Proteus mirabilis* (66.67%) than that of *Escherichia coli (*54.83%*).* The t-test result from Table 4 shows that there was no significant difference in the effect of

Cefepime on *Escherichia coli* (Mean value = 2.26) when compared with its effect on *Proteus mirabilis* (Mean value = 2.44) at $\alpha = 0.05$.

4. Discussions

Antimicrobial resistance surveillance is essential for effective control of infections and management. In the study, we investigated 9(22.50%) *Proteus mirabilis* and 31(77.50%) *Escherichia coli* isolates from clinical samples collected between March and August, 2013. Out of the forty bacterial isolates tested against cefepime, 17(54.83%) of *E. coli* and 6 (66.67%) of *Proteus mirabilis* were resistance to the antibiotic, this resistance to a fourth-generation in cephalosporins has increased for both *E. coli* and *Proteus mirabilis*. The susceptibility rate for *E. coli* and *Proteus mirabilis* in this study is 29.03% and 22.22% respectively. The susceptibility rate of *E coli* from this study 29.03% is higher than that of Kader and Kumar [24] who had 22.8% susceptibility rate. The resistance of *E coli* (54.83%) to cefepime is higher compare to that reported by [25] which in 2005 there was 1.7% resistance rate and 0.1% in 2002. In addition, resistance of clinical isolates to the critically important antibiotic cefepime, a member of fourth generation of cephalosporins, was observed in this study.

Table 1: Distribution of *Escherichia coli* and *Proteus mirabilis* from different Isolation Sites

Isolation Site	***Escherichia coli***	***Proteus mirabilis***	**Total (%)**
Wound	5	8	13(32.50)
Blood	1	0	1(02.50)
Ear	0	1	1(02.50)
High Vaginal	2	0	2(05.00)
Urine	16	0	16(40.00)
Sputum	0	0	0(00.00)
Abscess	1	0	1(02.50)
Catheter	1	0	1(02.50)
Stool	2	0	2(05.00)
Eye	2	0	2(05.00)
Cerebrospinal Fluid	0	0	0(00.00)
Throat	0	0	0(00.00)
Aspirate	1	0	1(02.50)
Total (%)	**31(77.50)**	**9(22.50)**	**40(100)**

Results showed that the greatest number of *Proteus mirabilis* isolates from clinical specimen were from wound and representing about 61.54% of all clinical samples from wound. Wounds recorded the highest percentage of *Proteus mirabilis* and *this* confirms the findings of [26] in Ghana and [27] in Nigeria who found that *Proteus mirabilis* is common in wound infections. This seems that *Proteus mirabilis* is more common or associated with

wound infections. While the highest isolates for *E coli* was from urine samples (16 were *E coli* out 31 isolates) compared to other clinical sites.

With no significant different in the effect of cefepime on the two bacterial species according to the t-test disagrees with the report of [28] sthat *E. coli* is susceptible to quinolones and many other antibiotics; but the result agrees with the study by [29], who noted that *Proteus* is characterized by a statistically limited presence of virulence factors but a multi-drug resistance pattern. The resistant of the clinical isolates to cefepime could be as a result of abuse of the antibiotics and there should be proper monitoring by qualified personnel in the field to curb the trends of antibiotics misuse.

Table 2: Susceptibility of *Escherichia coli* and *Proteus mirabilis* to Cefepime using CLSI (2007) Criteria.

Isolation site	*Escherichia coli*				*Proteus mirabilis*				Grand Total			
	S	**I**	**R**	**Total**	**S**	**I**	**R**	**Total**	**S**	**I**	**R**	**Total (%)**
Wound	1	2	2	**5**	2	1	5	**8**	3	3	7	**13(32.5)**
Blood	0	0	1	**1**	0	0	0	**0**	0	0	1	**01(2.5)**
Ear	0	0	0	**0**	0	0	1	**1**	0	0	1	**01(2.5)**
High vaginal	0	1	1	**2**	0	0	0	**0**	0	1	1	**02(5)**
Urine	7	1	8	**16**	0	0	0	**0**	7	1	8	**16(40)**
Sputum	0	0	0	**0**	0	0	0	**0**	0	0	0	**00(0)**
Abscess	0	0	1	**1**	0	0	0	**0**	0	0	1	**01(2.5)**
Catheter	0	1	0	**1**	0	0	0	**0**	0	1	0	**01(2.5)**
Stool	0	0	2	**2**	0	0	0	**0**	0	0	2	**02(5)**
Eye	1	0	1	**2**	0	0	0	**0**	1	0	1	**02(5)**
CSF	0	0	0	**0**	0	0	0	**0**	0	0	0	**00(0)**
Throat	0	0	0	**0**	0	0	0	**0**	0	0	0	**00(0)**
Aspirate	0	0	1	**1**	0	0	0	**0**	0	0	1	**01(2.5)**
Total (%)	**9(29.03)**	**5 (16.13)**	**17(54.83)**	**31(100)**	**2 22.22)**	**1(11.11)**	**6(66.67)**	**9(100)**	**11(27.50)**	**6 (15.00)**	**23 (57.50)**	**40 (100)**

Key: CLSI, (2007) Susceptibility Criteria for Cefepime; R≤ 14mm or I= 15-17mm or S≥ 18mm

S- Sensitive; I- Intermediate; R- Resistance.

Table 3: T- test for the effect of Cefepime on *Escherichia coli* and *Proteus mirabilis*

Organism	Effect Mean value	t-value
Escherichia coli	2.26	0.553
Proteus mirabilis	2.44	0.557

$p \leq 0.05$

5. Study Limitations

The data collected were limited to Ogbomoso Township in Oyo State of Nigeria; for a period of six months, there was a challenge of number of bacterial isolates obtained for that period.

6. Conclusion

There was a great resistance of Cefepime to the clinical isolates of *Escherichia coli* and *Proteus mirabilis*used in the study.

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