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Full Length Research Paper

Prevalence and antibiogram of Pathogenic Gram Negative Bacteria Isolated from patients Seeking Medical Care in a Tertiary Hospital in Abakaliki, Nigeria

Iroha Ifeanyichukwu¹, Ikeagwu Ifeoma², Ejikeugwu Chika^{1*}, Nwakaeze Emmanuel¹, Eze Egwu², Alu-OKafor Florence², Ugbo Emmanuel¹, Ikechukwu Moses¹ and Nwuzo Agabus¹

¹Department of Applied Microbiology, Faculty of Sciences, Ebonyi State University, P.M.B. 053, Abakaliki, Ebonyi State, Nigeria

²Department of Microbiology, Federal Teaching Hospital, Abakaliki, Ebonyi State, Nigeria

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Gram-negative bacteria are a heterogeneous group of bacteria that comprises the family *Enterobacteriaceae* and other non-enteric bacteria like *Pseudomonas aeruginosa*, and these organisms are responsible for a plethora of nosocomial and non-nosocomial infections. Antimicrobial resistance in Gram-negative bacteria is increasing at an alarming rate in both the hospital and community settings; and these organisms are responsible for a handful of infections in humans. This study evaluated by standard bacteriological analysis; 541 clinical samples of patients (who attended a tertiary hospital in Abakaliki, Nigeria) including urine, stool, sputum, eye swab, wound swab, high vaginal swab, and urethra swab. *Escherichia coli*, *Klebsiella* species, *Citrobacter* species, *Pseudomonas* species, *Morganella* species, *Proteus* species, and *Providencia* species were the Gram-negative pathogens isolated from the clinical samples. However, *E. coli* and *Klebsiella* species were the most prevalent Gram-negative bacteria isolated. All the Gram-negative bacteria showed 100 % resistance to sulphamethoxazole-trimethoprim and ampicillin. Resistance to ceftazidime, cefotaxime, amoxicillin-clavulanic acid, and ticarcillin-clavulanic acid was also common amongst the Gram-negative bacteria. All the Gram-negative bacteria showed 100 % susceptibility to the aminoglycosides, gentamicin and amikacin. Nitrofurantoin was only active against *E. coli*, *Citrobacter* species, *Providencia* species, *Morganella* species and *Klebsiella* species. Nevertheless, nitrofurantoin resistance was recorded in *Enterobacter* species, *Pseudomonas* species and *Proteus* species. Conclusively, the high level of antibiotic resistance observed in this study reveal that antimicrobial resistance among nosocomial bacterial pathogens is a worrisome development that warrants continuous surveillance and review of the usage of antimicrobial agents in our hospitals so that development and spread of drug-resistant organism can be effectively contained.

Keywords: Gram-negative bacteria, Nosocomial infection, Antimicrobial resistance, Hospital patients

INTRODUCTION

The ability of pathogenic bacteria to become resistant to antibiotics is a growing public health issue that is

gradually affecting the treatment outcome of patients; and the presence of resistant strains of bacterial pathogens results in prolonged illnesses and higher mortality and morbidity rates. The use of antibiotics encourages the emergence of resistant bacteria, and this phenomenon is worse when drugs are used irrationally and without proper susceptibility test results (Jayaramah, 2009;

*Corresponding Author E-mail: ejikeugwu_chika@yahoo.com;
Phone: +2348097684562

Okonko *et al.*, 2009). The continued prevalence of multidrug resistant bacteria reported in several strains of *Enterobacteriaceae* (e.g. *Escherichia coli* and *Klebsiella* species) and other non-enteric bacteria like *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is worrisome and portends important risk regarding clinical diagnosis (Ejikeugwu *et al.*, 2009; Adeleke *et al.*, 2010; Sanjay *et al.*, 2010; Quinn *et al.*, 1989; Philip *et al.*, 1997). As one study puts it, the intense use and misuse of antimicrobial agents are undoubtedly the major forces associated with the high numbers of resistant pathogenic and commensal bacteria worldwide (Barbosa and Levy, 2000). Gram negative bacteria have unique cell walls that are different from Gram positive bacteria, and the presence of outer membrane (OM) and other physiological barriers such as their innate influx-efflux mechanisms allows them to be readily resistant to antimicrobial agents (Brooks *et al.*, 2004; Madigan *et al.*, 2009). Gram-negative bacteria are notorious in causing nosocomial infections, and they are also implicated in a number of community-acquired infections too. These organisms are able to thrive even in the phase of antimicrobial onslaught because they produce a wide variety of beta-lactamase enzymes that allows them to evade the action of some available antimicrobial agents (Eze, 2012; Okonko, *et al.*, 2009). The determination of bacterial resistance to antimicrobials is an important part of the management of infections in patients, and this procedure should be carried prior to any meaningful antimicrobial therapy. The problem of antibiotic resistance is a vital topic and a global health problem that has received increasing attention over the last two decades. It is certainly not a new topic neither was it unpredictable (Mascaretti, 2003). And when antibiotic resistance occurs, it is the microbe (bacterium) that is resistant, not the antibiotic nor the patient or host taking it. Bacterial resistance has evolved with an increased number, volume and diversity of antimicrobial applications (Barbosa and Levy, 2000). Thus, there is need for regular and accurate determination of the antibiogram of pathogenic bacteria from clinically important specimens prior to the prescription of antimicrobial agents in order to guide therapy.

MATERIALS AND METHODS

Study Area: The study area was Federal Teaching Hospital, Abakaliki (FETHA), Ebonyi state, Nigeria. Clinical isolates of Gram negative bacteria was isolated from clinical samples of patients presented for diagnosis in the Microbiology Laboratory Unit of Federal Teaching

Hospital Abakaliki.

Collection of Clinical Samples: A total of five hundred and forty-one (541) clinical samples were collected from different hospital wards and outpatients of Federal Teaching Hospital Abakaliki in Ebonyi State. The samples include urine, stool, sputum, eye swab, wound swab, high vaginal swab, and urethra swab. They were analyzed at the Applied Microbiology laboratory unit of Ebonyi State University Abakaliki by culture, Gram staining, biochemical test and antibiotics sensitivity testing (Cheesbrough, 2000).

Bacteriological analysis of clinical samples: All the clinical samples were aseptically inoculated onto culture media (prepared according to the manufacturers instruction) including blood agar, chocolate agar, MacConkey agar, Cysteine lactose electrolyte deficiency agar (CLED), *Salmonella-Shigella* agar (SSA), and deoxycolate citrate agar (for stool samples). The culture media plates were incubated aerobically at 37°C for 18 - 24 hrs; and bacterial growths were microscopically and macroscopically observed for colony appearance and other growth patterns unique to each isolated organisms. Suspected cultures were sub-cultured onto freshly prepared culture media plates as aforementioned for the isolation of pure cultures; and the isolates were identified based on standard morphological and biochemical tests including Gram staining, citrate test, urease test, voges proskauer test, indole test, catalase test, methyl red test and hydrogen sulphide production test (Cheesbrough, 2000). Sugar fermentation test was performed using triple sugar iron agar (TSIA) for identifying organisms that fermented glucose, sucrose and lactose.

Antibiogram: Antimicrobial susceptibility testing was carried out by the Kirby-Bauer disk diffusion method as per the criteria of National Committee for Clinical Laboratory Standards (NCCLS) using single antibiotic disks of ampicillin (10 µg) sulphamethoxazole/trimethoprim (30 µg), nitrofurantoin (15 µg), cefotaxime (30 µg), ciprofloxacin (10 µg), amikacin (10 µg), gentamicin (10 µg), ofloxacin (10 µg), ceftazidime (30 µg), amoxicillin/clavulanic acid (30 µg) and cefuroxime (30 µg) (Oxoid, UK) as was previously described (Ejikeugwu *et al.*, 2013; Okonko *et al.*, 2009; NCCLS, 1999). A suspension of the test isolate (adjusted to 0.5 McFarland turbidity standards) was aseptically streaked on Mueller-Hinton agar plates, and the individual antibiotics were aseptically placed on the inoculated plates. Plates were allowed for 10 mins and then incubated at 37°C for 24 hrs. The inhibition zone diameter (IZD) produced by the drugs were taken and reported according to the NCCLS guidelines (NCCLS, 1999).

Sample Distribution

- Urine
- Stool
- Endocervical swab
- High vaginal swab
- Wound swab
- Semen
- Sputum
- Cerebrospinal fluid
- Eye swab
- Throat swab
- Urethral swab
- Ear swab

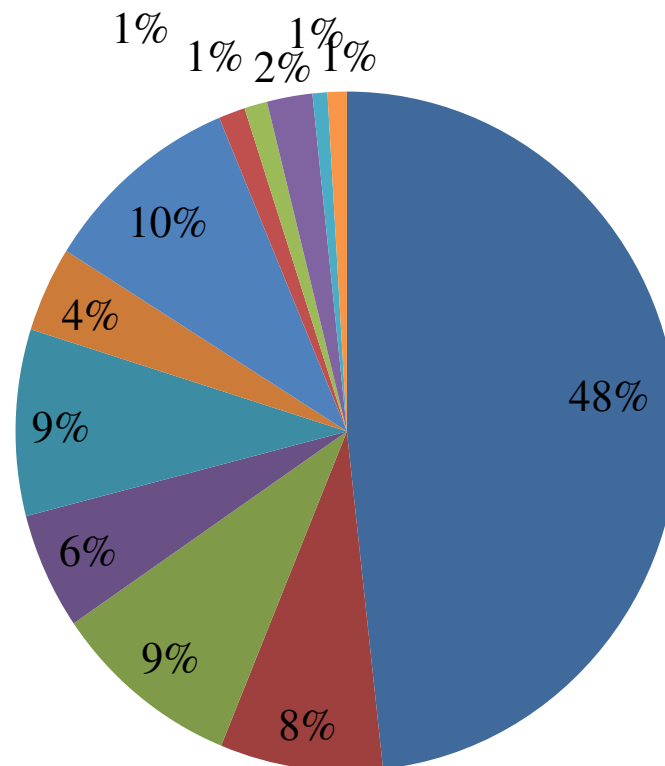


Figure 1. Distribution of the clinical samples

RESULTS

The distribution of clinical samples recruited for this study is shown in Figure 1. More samples were collected from patients with urinary tract infections (261 urine samples), gastrointestinal infections (43 stool samples, patients with burn/wound infections (48 wound swabs), patients with respiratory tract infections (53 sputum samples) and patients with one form of genital infection or the other including high vaginal swab (30 HVS samples), endocervical swab (50 ECS), and semen samples (22). Ear swab samples (7), urethral swab samples (4), throat swab samples (12), and eye swab samples (6) were also included in this study (Figure 1). The Gram negative bacteria isolated from the clinical specimens are bacterial species of the genera *Enterobacter*, *Morganella*,

Pseudomonas, *Providencia*, *Klebsiella*, *Escherichia*, *Proteus* and

Citrobacter (Table1). While some of the clinical samples including urine samples, stool, endocervical swab (ECS), high vaginal swab (HVS), ear swab and wound swab showed significant growth on culture media, the other clinical samples that included cerebrospinal fluid (CSF), eye swab, throat and urethral swab produced no significant bacterial growth. Only one bacterial genera was recovered from CSF samples (*Pseudomonas* species) and sputum samples (*Klebsiella* species) respectively (Table 1). The most commonly isolated Gram-negative bacteria were *Escherichia coli*, *Klebsiella* species, *Citrobacter* species, *Pseudomonas* species, *Proteus* species, and *Providencia* species.

Table 1. Frequency of isolation of Gram-negative bacteria from the clinical samples

Sample	<i>Citrobacter</i> spp	<i>Proteus</i> spp	<i>Escherichia coli</i>	<i>Klebsiella</i> spp	<i>Providencia</i> spp	<i>Pseudomonas</i> spp	<i>Morganella</i> spp	<i>Enterobacter</i> spp
Urine	6	5	11	9	3	2	1	1
Stool	0	1	0	0	0	0	0	0
ECS	2	0	4	1	0	0	0	0
HVS	1	4	3	0	0	0	0	0
Wound	4	0	1	1	0	5	0	1
Semen	0	0	0	0	0	0	0	0
Sputum	0	0	0	1	0	0	0	0
CSF	0	0	0	0	0	1	0	0
Eye Swab	0	0	0	0	0	0	0	0
Throat swab	0	0	0	0	0	0	0	0
Urethral Swab	0	0	0	0	0	0	0	0
Ear Swab	0	0	1	1	0	0	0	0
Total	13	10	20	13	4	8	1	2

ECS= endocervical swab, HVS= high vaginal swab, CSF= cerebrospinal fluid

Table 2. *In vitro* antimicrobial susceptibility of the Gram negative bacteria

Organism	CIP	AMC	CAZ	AK	SXT	CN	OFX	AMP	TIM	F	CTX
<i>Citrobacter</i> spp	S	R	R	S	R	S	S	R	R	S	R
<i>Proteus</i> spp	R	S	S	S	R	S	R	R	S	R	S
<i>Escherichia coli</i>	S	R	R	S	R	S	R	R	R	S	R
<i>Klebsiella</i> spp	S	S	R	S	R	S	S	R	S	S	R
<i>Providencia</i> spp	S	R	R	S	R	S	S	R	R	S	S
<i>Pseudomonas</i> spp	S	R	R	S	R	S	S	R	R	R	R
<i>Morganella</i> spp	S	R	R	S	R	S	S	R	R	S	S
<i>Enterobacter</i> spp	S	R	R	S	R	S	S	R	R	R	R

CIP – Ciprofloxacin, AMC – Amoxicillin-clavulanic acid, CAZ – Ceftazidime, AK – Amikacin, SXT – Sulphamethoxazole-trimethoprim, CN – Gentamicin, OFX – Ofloxacin, AMP – Ampicillin, TIM - Ticarcillin-clavulanic acid, F – Nitrofurantoin, CTX – Cefotaxime.

The antimicrobial susceptibility test results of the isolated Gram negative bacteria to some commonly available antibiotics including ciprofloxacin, amoxicillin-clavulanic acid, ceftazidime, amikacin, sulphamethoxazole-trimethoprim, gentamicin, ofloxacin, ampicillin, ticarcillin-clavulanic acid, nitrofurantoin, and cefotaxime is shown in Table 2. The antibiotic susceptibility studies reveal that all the test isolates were resistant to sulphamethoxazole-trimethoprim, ceftazidime, ampicillin, ticarcillin-clavulanic acid and cefotaxime. However, some level of susceptibility of the test isolates to ciprofloxacin, ofloxacin, nitrofurantoin, and amoxicillin-clavulanic acid was also recorded (Table 2).

DISCUSSION

Antimicrobial resistance is not a new problem in the healthcare system but the menace is fast becoming a serious issue – owing to the rate at which some antimicrobial agents are rendered in efficacious (*in vivo*) when used for treatment. Though a natural phenomenon associated with the evolution of microorganisms (bacteria in particular), antibiotic resistance limits the lifespan of our antibiotics; and the unnecessary and irrational use of antibiotics favours the emergence and spread of resistant bacteria in both the hospital and community settings. This study evaluated the antibiogram of Gram-negative

bacteria isolated from various clinical samples from Federal Teaching Hospital Abakaliki, Nigeria. Antimicrobial susceptibility studies are important in the proper care of a sick patient because it measures the ability of different antibiotics to inhibit or kill a given pathogenic bacterium *in vitro*. And the data from such studies gives the physician clues as to the proper type of drugs to select for the treatment of the sick patient. *Escherichia coli*, *Klebsiella* species, *Citrobacter* species, *Pseudomonas* species, *Morganella* species, *Proteus* species, and *Providencia* species were the Gram-negative pathogens isolated from the various clinical samples recruited for this present day study. *E. coli* and *Klebsiella* species were the most commonly isolated bacterial pathogens from the clinical samples. Several studies has reported the prevalence of *E. coli* and *Klebsiella* species as important causative factors of a handful of bacterial-related infections in humans (Varghese *et al.*, 2010; Okonko *et al.*, 2009; Sanjay *et al.*, 2010). And these Gram-negative organisms have also been reported as carriers of some high profile antibiotic hydrolyzing enzymes such as extended spectrum beta-lactamases and metallo-beta-lactamases (Philip *et al.*, 1997; Jacoby and Munoz-Price, 2005; Ejikeugwu *et al.*, 2013). The Gram-negative bacteria we isolated in this study are responsible for several community-acquired and nosocomial infections. The antibiogram result showed that all the Gram-negative bacteria (*Escherichia coli*, *Klebsiella* species, *Citrobacter* species, *Pseudomonas* species, *Morganella* species, *Proteus* species, and *Providencia* species) isolated in our study were highly resistant to sulphamethoxazole-trimethoprim and ampicillin. The Gram-negative bacteria were also resistant to the 3rd-generation cephalosporins ceftazidime and cefotaxime. Reduced susceptibility of Gram negative bacteria to these antibiotics (as obtainable in this study) have also been reported by other researchers (Adeleke *et al.*, 2010; Varghese *et al.*, 2010). All the Gram-negative bacteria were highly susceptible to the aminoglycosides, gentamicin and amikacin as shown in Table 2. The fluoroquinolones, ciprofloxacin and ofloxacin also showed moderate levels of activity against the test Gram-negative pathogens excluding *Proteus* species and *E. coli* that were both resistant to ciprofloxacin and ofloxacin respectively. *E. coli* was also resistant to ceftazidime, cefotaxime and ofloxacin in this study. Nkang *et al.* (2009) also reported similar high profile resistance of Gram-negative bacteria to 3rd-generation cephalosporins and the fluoroquinolones. Okonko *et al.* (2009) and Adeleke *et al.* (2010) also high levels of resistance amongst Gram negative bacteria to most of the antibiotics used in this study. The Gram-negative bacteria were also highly resistant to the beta-lactamase inhibitors, amoxicillin-clavulanic acid and ticarcillin-

clavulanic acid. The high level of resistance recorded across the Gram negative bacteria isolated in this study is worrisome. This is because these organisms are responsible for several bacterial-related infections in humans; and if proper susceptibility study is not carried out in the course of treatment, the prognosis of the patients undergoing treatment might come out poor. And it could be deduced from our presumptive study that the hospital under study could be a potential reservoir of nosocomial pathogens that express different levels of drug resistance factors, genes and or enzymes that renders antibiotics less-efficacious in treatment. Conclusively, this present day study showed that *Escherichia coli*, *Klebsiella* species, *Citrobacter* species, *Pseudomonas* species, *Morganella* species, *Proteus* species, and *Providencia* species were the most prevalent Gram negative pathogens isolated from clinical samples of patients in the tertiary hospital under study; and these organisms are multiple resistant to some commonly used antibiotics especially ampicillin, sulphamethoxazole-trimethoprim, nitrofurantoin, ofloxacin amoxicillin-clavulanic acid and ticarcillin-clavulanic acid.

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