

THE SUSCEPTIBILITY OF BACTERIA ISOLATES FROM PARTS OF THE BODY TO ANTIBACTERIAL AGENTS AT THE UNIVERSITY OF BENIN TEACHING HOSPITAL (U.B.T.H), BENIN CITY, EDO STATE, NIGERIA

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ABSTRACT

Nigeria, like most developing countries of the world has a high degree of antibiotic resistance emanating from antibiotic selective pressure coupled with antibiotic abuse. This study aims to determine the resistance pattern of bacterial isolates from various parts of the body in a tertiary healthcare institution. The study which was prospective and cross-sectional lasted one year, during the period from June 2008 to May 2009. Swabs and aspirates were obtained from patients who were seen at the various facilities in the hospital. Samples were cultured and incubated at 37°C both aerobically and anaerobically for 24 hours to 48 hours. The various media for culture were Chocolate, Blood and McConkey agars. Antibiotic susceptibility test was done on nutrient agar using the agar diffusion method of Bauer and Kirby.

The bacterial isolates include *Escherichia coli* (10.6%), *Klebsiella pneumoniae* (10.6%), *Pseudomonas aeruginosa* (10.3%), *Proteus vulgaris* (3.0%), *Proteus mirabilis* (12.6%), *Proteus rettgeri* (0.6%) *Morganella morganii* (1.2%) *Providencia stuartii* (2.3%) *Strept. Pyogenes* (0.3%), *Alkaligenes faecalis* (3.0%). All the isolates were susceptible to the quinolones and the Cefuroxime except *Pseudomonas aeruginosa* and *Strept. pyogenes*. The later showed some sensitivity to amoxicillin, erythromycin and amocillin/ clavulanate while other isolates were strongly resistant to Cloxacillin, tetracycline, cotrimoxazole and chloramphenicol. *E.coli*, *P. mirabilis* and *Morgenella morgani* were susceptible to gentamycin while other isolates were resistant to it.

Introduction

The discovery of potent antibiotics was one of the greatest contributions to healthcare delivery in the 20th century¹. However, the emergence of resistance to these antimicrobial agents became a threat to the

advances in health care delivery. The incidence of resistance is multifactorial. It can be a result of horizontal gene transfer² or a result of unlinked point mutation in the pathogen genome at a rate of about 1 in 10⁸ per chromosomal mutation³. The antibiotic action against the pathogen can be seen as an environmental pressure. Any population of organisms, bacteria included, naturally includes traits such as the ability to resist antibiotic attack⁴. The drug kills the defenseless bacteria, leaving behind or "selecting" those that can resist it. The selected bacteria multiply, increasing their number a million fold in a day and become the predominant organisms.

KEY WORDS: Bacterial isolates, Antibiotic, Resistance, Bacterial Infections.

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Antibiotics do not technically cause resistance but allow it to happen by creating a situation where an already existing variant can flourish³. Whenever antibiotics are used there is a selective pressure for resistance to occur. It builds upon itself and more organisms develop resistance to more drugs⁴ Some microbes have the ability to acquire resistance plasmid horizontally, while some use the efflux mechanism to prevent antibiotics attaining a lethal concentration in the microbe. Disease-causing microbes have developed some weaponry by which they thwart antibiotics by interfering with their mechanism of action. For example penicillin kills by attaching to the cell wall, then destroy a key part of the wall. The wall falls apart and the bacterium dies. Resistant microbes either alter their walls so that penicillin cannot bind or produce enzymes that dismantle the antibiotics. In another example, erythromycin attacks ribosome and disrupts the ability of the organism to make proteins. Resistant organisms have slightly altered ribosome to which the drug cannot bind. During the 2nd world war when penicillin became widely available it was a medical miracle rapidly destroying the biggest wartime killer, the infected wounds. It was scarcely four years after drug companies began mass production of penicillin that microbes started appearing that could resist it³. The first bug to resist penicillin was *Staphylococcus aureus*. The organism is normally a harmless passenger in the body, but can cause illness such as pneumonia or toxic shock syndrome when it overgrows or produces a toxin⁵.

There are abundant reports in the literature

on the sensitivity of bacteria to antimicrobial agents^{2,6,7-15}. As the prevalence of resistance to antimicrobial agents continues to rise, there is need to have a high quality surveillance system which will play a major role in combating emergence of antibiotic resistance. There must be surveillance at local level to provide accurate, up to date information on local susceptibility patterns¹⁶.

Since emergence of resistance is more or less, a local problem, surveillance is strongly recommended in each geographical area on a regular basis to monitor the emergence of resistance to commonly used antimicrobial agents. The need therefore, to monitor antibiotic resistance in a tertiary healthcare institution can not be over emphasized. In this study the sensitivity pattern of the various bacterial isolates from different sites of the body was highlighted.

Materials and Methods

This study was conducted at the University of Benin Teaching Hospital, a 600-bed referral and tertiary care facility located in Benin City, Edo state Nigeria. Benin city is a cosmopolitan city and capital of Edo State.

Specimens were taken from patients who were seen at the various facilities in the hospital in the form of swabs and aspirates from various parts of the body. They consisted of urethra, endocervix, higher vagina, ear, eye, throat and wound swabs. Aspirates and secretions were also examined. There was no particular selection order except that they were consecutively obtained. 281 samples were examined. 120 (42.7%) were females while

161 (57.3%) were males. The samples consisted of 26 endocervical swabs, 131 wound swabs, 22 urethral swabs, 36 higher vaginal swabs, 47 ear swabs, 9 eye swabs, 5 aspirates, 4 throat swabs, and 1 catheter tip. The various samples required different culture media and techniques. The microbiological samples obtained from endocervix, higher vagina, urethra, throat, ear and eye were plated on chocolate agar, (Oxoid No Cm 271), blood agar (Oxoid No Cm 271) and McConkey agar (Oxoid No 7). Higher vagina and ear swabs required sabouraud agar in addition for detection of any fungal species. The wound swabs and the catheter tip required blood agar, McConkey agar and Robinson cooked meat, and Nutrient agar was used for sensitivity test¹⁷. With the exception of throat, eye and urethral swabs all were incubated both aerobically and anaerobically. All the samples inoculated on chocolate required 5% - 10% carbon dioxide condition. In this study the candle method was used to generate carbon dioxide.

Culture Technique: The various agar plates were streaked aseptically with sterile wire loop and well-spaced out to form discrete colonies. The inoculated plates were incubated at 37°C. The plates which required anaerobic incubation were put in anaerobic jar filled with hydrogen gas, while others were incubated aerobically. Grease free slides were smeared with each specimen for Grams staining. Some samples required direct wet microscopy for the detection and identification of parasitic ova and trophozoites. Lastly Robertson's cooked meat (RCM) was inoculated onto blood agar and McConkey agar after

overnight incubation and re-incubated. The blood agar plate was incubated both aerobically and anaerobically. Plates were examined after 24 hours, and those which did not have growth were reincubated for another 24 hours. All the isolates were identified using colonial morphology and biochemical reactions according to the methods of Cowan and Steel¹⁸.

Susceptibility test was done by the disc diffusion method, a modification of Bauer and Kirby.¹⁹ With a sterile forceps, commercially prepared antibiotic discs were placed at least 25mm apart on nutrient agar plates for aerobes and non fastidious organisms. Blood agar was used for fastidious organisms like *Streptococcus pneumoniae*.

The different antimicrobial agents used and their disc contents were-Peflacin 5mg; Sparfloxacin 5mg; Cefuroxime 30mg; Amoxicillin 25mg; Gentamycin 10mg; Cloxacillin 10mg; Erythromycin 10mg; Cotrimoxazole 25mg; Tetracycline 25mg; Ciprofloxacin 5mg; Amoxicillin clavulanate 10mg; and Chloranphenicol 10mg. Plates were incubated at 37°C for 24 hours, after which the zones of inhibition in each case were measured and compared with control organism. A standard sensitive strain *Escherichia coli* (Cw 3310) was included as the control organism. The zone of inhibition was measured to determine sensitive and non-sensitive organisms. "R" represents resistance, while "S", represents sensitivity to the antibiotic. Results were presented descriptively.

RESULTS

A total of 310 isolates were obtained and they comprised 287 bacteria and 23 candida species. The isolates comprised of

33 *Escherichia coli* (10.6%), 33 *Klebsiella Pneumoniae* (10.6%); 32 *Pseudomonas aeruginosa* (10.3%); 6 *Proteus vulgaris* (3%); 39 *Proteus mirabilis* (12.6%); 2 *Proteus rettgeri* (0.6%); 4 *Morganella morganii* (1.2%); 124 *Staphylococcus aureus* (40%); 7 *Providencia stuartii* (2.3%); 1 *Streptococcus pyogenes* (0.3%); 6 *Alkaligenes faecalis* (3%); 23 *Candida* species (7.4%). (Table I)

The sensitivity pattern of all isolates to the different antibiotics was shown in table ii. The *Candida* species were excluded from the sensitivity test since they are not known to respond to antibiotics. All the

isolates showed some degree of susceptibility to the quinolones, and cefuroxime. *Pseudomonas aeruginosa* and *Streptococcus pyogenes* were resistant to cefuroxime while *Streptococcus pyogenes* showed some sensitivity to amoxicillin, erythromycin, and amoxicillin clavulanate. All isolates showed strong resistance to cloxacillin, cotrimoxazole, tetracycline and chloramphenicol. Gentamycin was relatively effective against *Escherichia coli*, *Proteus mirabilis* and *Morganella morganii*. With the exception of these three organisms other isolates demonstrated strong resistance to gentamycin

Table i: shows the % occurrence of the various bacterial isolates species:

No. of Isolates	Percentages occurrence
<i>Escherichia coli</i>	33 (10.6%)
<i>Klebsilla pneumoniae</i>	33 (10.6%)
<i>Pseudomonas aeruginosa</i>	32(10.3%)
<i>Proteus mirabilis</i>	39(12.6%)
<i>Proteus vulgaris</i>	6(3%)
<i>Morganellamorganii</i>	4(1.2%)
<i>Proteus rettgeri</i>	2(0.6%)
<i>Streptococcus pyogenes</i>	1(0.3%)
<i>Staphylococcus aureus</i>	124(40%)
<i>Providencia stuartii</i>	7(2.3%)
<i>Alkaligenes faecalis</i>	6(2%)
<i>Candida</i> species	23(7.4%)

Table ii: Shows the antibacterial susceptibility of the clinical isolates antimicrobial agents

Isolates	NO	Pef	Sp	Cxm	Amx	CN	OB	E	SXT	Te	CIP	AUG	C
<i>Escherichia coli</i>	33	23 69.7%	26 78.8%	22 66.6%	2 6%	21 63.6%	2 6%	2 6%	Nil	1 3%	30 90.9%	6 18.2%	12 36.4%
<i>Klebsiella Pneumoniae</i>	33	25 75.8%	28 84.8%	17 51.5%	4 12.1%	13 39.4%	3 9.1%	2 6.1%	2 6.1%	1 3.0%	29 87.9%	10 30.3%	7 21.2%
<i>Pseudomonas aenuginosa</i>	32	17 53.1%	19 59.4%	10 31.3%	Nil	11 34.4%	Nil	Nil	Nil	Nil	28 87.5%	2 6.3%	Nil
<i>Proteus vulgaris</i>	6	3 50%	3 50%	4 66.7%	Nil	2 33.3%	Nil	Nil	Nil	Nil	5 83.3%	Nil	3 50%
<i>Proteus rettgeri</i>	2	1 50%	1 50%	1 50%	Nil	Nil	Nil	Nil	Nil	Nil	1 50%	Nil	1 50%
<i>Proteus mirabilis</i>	39	26 66.6%	27 69.2%	20 51.3%	Nil	21 53.8%	3 7.7%	1 2.6%	Nil	Nil	29 74.4%	4 10.3%	11 28.2%
<i>Morganella morganii</i>	4	3 75%	2 50%	2 50%	Nil	3 75%	Nil	Nil	Nil	Nil	3 75%	1 25%	1 25%
<i>Staphylococcus aureus</i>	124	75 60.5%	75 60.5%	92 74.2%	10 8.1%	60 48.4%	15 12.1%	35 28.2%	Nil	2 1.6%	106 85.5%	30 24.2%	42 33.9%
<i>Providencia stuartii</i>	7	6 85.7%	7 100%	4 57.1%	Nil	3 42.8%	Nil	Nil	Nil	Nil	6 85.7%	Nil	1 14.3%
<i>Streptococcus pyogenes</i>	1	Nil	Nil	Nil	1 100%	Nil	Nil	1 100%	Nil	Nil	1 100%	1 100%	Nil
<i>Alkaligenes faecalis</i>	6	4 66.7%	4 66.7%	3 50%	1 14.3%	Nil	Nil	Nil	1 14.3%	2 28.6%	4 66.7%	1 14.3%	1 14.3%
Total Isolate	287												

Footnote:

PEF= Perflacin
 CXM= Cefuroxime
 CN= Gentamycin
 E = Erythromycin
 TE=Tetracycline
 AUG= Amoxicilin clavulanate

SP= Sparfloxacin
 AMX= Amoxicillin
 OB=Cloxacillin
 SXT=Cotrimoxazole
 CIP= Ciprofloxacin
 C= Chloranphenicol

Discussion

This study clearly showed that *Staphylococcus aureus* is the predominant organism in swab specimens analyzed in UBTH within the survey period. This situation may not be unconnected with the ubiquity of *Staphylococcus aureus* as it colonizes body surfaces and also the invaginations which comprise the nostrils, mouth, anus, vagina and the urethra¹⁹. The colonization of these areas predisposes the individual to *Staph aureus* infection²⁰.

Although some staph strains are usually harmless as they are commensals, however, injury or break in the skin enables the organism to invade the body and overcome the body's natural defenses. The consequences can range from minor lesions to deep-seated infections^{21,22}.

Staphylococcus aureus is a hardy bacterium as it was shown in a study where it survived for three months on a piece of polyester, a material, being the main material used in hospital privacy curtains²³. The presence of staph organism on hospital equipments makes it rank as a nosocomial pathogen²⁴. *Proteus mirabilis* was next numbering 39(12.6%). This organism is a member of the family enterobacteriaceae and is usually found in the human gut. It is an organism that is often considered to be involved in contamination and colonization, and is occasionally isolated in severe infections²⁵.

The clinical relevance of *Proteus mirabilis* has been demonstrated since 1990 in the increase in frequency of resistance to beta-lactams, aminoglycosides and the quinolones²⁶. This is one of the gram-

negative organisms that are implicated in nosocomial infections. *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* occurred at almost the same frequency. Their clinical relevance lies in the fact that they are the gram-negative organisms which have the capability of producing extended spectrum beta-lactamases (ESBLs)²⁷. Some of them have efflux pump system which confers resistance to antibiotics²⁸. The drugs are pumped out so that they can not attain the lethal level in the body of the organism. The frequency occurrence of the other isolates is too insignificant to be of any clinical relevance.

The ultimate goal of bacteria and other microbes is to reproduce and multiply and for this reason they spread to acquire uncolonised territories; that is to acquire new hosts. This spread is presently enhanced by some societal factors such as increase in human population and increased national and international transport networks. Today the world population has grown to over 6 billion (United Nations report)²⁹ and more people are traveling greater distances both nationally and internationally for reasons of trade, tourism and migration.

Traveler-carried resistant pathogen can be easily transported beyond its site of emergence to other countries or continents even before the emergence of the resistance is detected.

This study demonstrated the increased emergence of resistance to commonly used antimicrobial agents in University of Benin Teaching Hospital as evidenced by the

antibiogram of bacterial isolates. Apart from the fluorinated quinolones and cefuroxime, the bacterial isolates, were strongly resistant to the other antimicrobial agents. The organisms strongly resisted cotrimoxazole and tetracycline and exhibited the same resistance to amoxicillin, amoxicillin clavulanate, chloramphenicol and erythromycin. Other workers have reported with concern the emergence of macrolide resistance among *Streptococcus pyogenes* and as in many regions, exceeds the resistance to penicillin¹⁴. However the only one isolate of *Streptococcus pyogenes* in the study was sensitive to erythromycin but it can not be used to determine the susceptibility of the organism to erythromycin. Some workers have reported that resistance to the fluoroquinolones is still relatively rare in many parts of the world³⁰.

However, at the University of Benin Teaching Hospital, resistance to the fluoroquinolones is real as can be seen in the antibiogram of the bacterial isolates. This means that the prevalence of resistance may depend on local factors, which vary from one geographical location to another.

Antibiotic resistance arises because pathogens undergo evolutionary process i.e. natural selection which brings about alteration in phenotype and / or genotype meaning the antibiotic can no longer target them⁴. This often arises because people fail to finish their course of antibiotics. They stop when they are feeling better. There can be a few bacteria left over which develop resistance and proliferate faster than those which are still susceptible.

Therefore resistance spreads very fast. Unfortunately many doctors prescribe antibiotics for complaints that could be best treated with aspirin and when an antibiotic is essential the effect is negative due to their excessive use that generates resistance. Too many pharmacies serve antibiotics across the counter and so many people are overdosing themselves. The more we use antibiotics and over use them, the more the bugs they kill develop defenses⁶.

Staphylococcus aureus, which is the predominant bacterial isolate in the study showed a high degree of resistance to the antimicrobial agents. It was only relatively sensitive to the quinolones specifically Ciprofloxacin and also to Cefuroxime. It was resistant to the other antibiotics. Cloxacillin which is regarded as drug of choice for *Staphylococcus aureus* infections was only 12.1% susceptible to the drug. This is a worrisome situation because of the ubiquity of *Staphylococcus aureus* all over the human skin and the many sources of infection. The consequence is that in serious *Staphylococcus aureus* infections clinicians will have very limited antibiotic options. The bacteriology of this study is not in consonance with previous works in which the gram negative organisms had always been the predominant organisms especially the members of enterobacteriaceae^{31,32}. The penicillins and the cephalosporins which had been effective for the treatment of *Staphylococcus aureus* infections have been rendered ineffective as a result of either penicillinase production or mutation due to drug selective pressure. The other bacterial isolates in the study showed the same resistance pattern.

CONCLUSION:

This study has provided evidence to show the increasing rate of bacterial resistance to antimicrobial agents, and this constitutes a big challenge to clinicians in the management of infections. This trend in antibiotic resistance gives cause for concern especially in developing countries where these drugs are used as first line drugs. The easy access of antibiotics in shops and open markets as well as the consumption of drugs without doctor's prescription should be discouraged. Routine sensitivity testing of clinical isolates to antibiotics before prescription should be encouraged.

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