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# Peculiar pattern of antibiotic resistance in bacteria isolated from various sources in South-East Nigeria and the implications in health and economy

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**ABSTRACT:** The antimicrobial susceptibilities of bacteria isolated from various sources in the South- East of Nigeria were studied with the aim of providing data on resistance patterns as well as highlighting any implications of the findings to health and economy. The antibiotic susceptibilities of bacteria isolates including *Escherichia coli* (n=6), *Pseudomonas aeruginosa* (n=9), a typed sample of *Pseudomonas aeruginosa* ATCC 10145, *Salmonella typhi* (n=3), *Klebsiella pneumoniae* (n=1), *Shigella dysenteriae* (n=1), *Staphylococcus aureus* (n=5) and *Streptococcus pneumoniae* (n=1) were determined using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar. The findings revealed that all the isolates showed multiple antibiotic resistances ranging from 30% to 100%, intermediate resistance 0%-20% and susceptibility 9.75-60%. Five (18.4%) isolates showed 'pan-resistance' (100%). The highest resistance (100%) was shown to ampicillin, nalidixic acid, augmentin, trimethoprim-sulfamethoxazole and cephaplexin. The highest susceptibility was shown to levofloxacin (100%), ofloxaxin (66.7%), ciprofloxacin (60%) and perfloxacin (50%). The findings support the claim that ciprofloxacin is still the most effective second line broad spectrum antibiotic. The implications of drug resistance in health and economy, which include higher mortality rates, longer duration of illness and treatment, increased health-care costs and economic burden, the resort to alternative herbal treatments which further compound health problems and the emergence of 'pan-resistant' bacterial pathogens were highlighted. Proffered solutions include appropriate use of antibiotics by health workers, concerted efforts to control procurement and use of antibiotics, and the implementation of a regional and nationwide surveillance system to monitor antimicrobial resistance trends in Nigeria. © JASEM

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Antibiotic resistance has caused a high mortality rate as well as great economic losses in Nigeria and globally (Chukwuma, 2011). Powerful antibiotics first became commercially available in the 1940s and have saved untold millions of lives. The first marketed modern antibiotic was Penicillin, discovered by Fleming in 1928. At the end of the 2nd world war, penicillin became widely available and won widespread acceptance. Soon after, resistance to penicillin was observed in bacteria. In his 1945 Nobel Prize lecture, Fleming himself warned of the danger of resistance – "It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body……." It took little time for *Staphylococcus aureus* to develop resistance to penicillin. In 1947 physicians observed the first case of clinical resistance. With few exceptions, each introduction of a new antibiotic has been followed within a few years by the first cases of resistance. Resistance then spreads. In addition, more variations of resistance mechanism against the same drug may also continue to emerge over time, compounding the problem (Milatovic and Breveny, 1987).

According to World Health Organization (WHO) annual report titled "Overcoming Antimicrobial Resistance", almost all major infectious diseases are slowly but surely becoming resistant to existing medicines (World Health Organization, 1988). *Pseudomonas aeruginosa* is the most notorious of all the resistant pathogens with many multi-resistant strains. Strains of *P. aeruginosa*, as well as *S. aureus*, *Salmonella typhi* and *Escherichia coli* have shown increasing resistance to all classes of antibiotics, especially the first and second generation antibiotics. By 1993, multiple-drug-resistant *S. typhi* were isolated in Viet Nam. They were resistant to the usual first line antibiotics: chloramphenicol, ampicillin and

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co-trimoxazole but fully susceptible to fluoroquinolones (Wain et al., 1997). *E. coli* is inherently susceptible to cephems, but with increased use of broad spectrum cephalosporins, cephem-resistant *E. coli* isolates are now commonly encountered in various clinical settings (Yagi et al., 1997). By 2003, the emergence of *P. aeruginosa* resistance to quinolones was observed, and has been reported to be on the increase (Olayinka et al., 2004).

Antimicrobial resistance is a natural phenomenon, amplified manifold due to human misuse and neglect of antimicrobial drugs. The situation of antimicrobial resistance appears to be due to a combination of factors which include socio-economic status, overuse of antibiotics in food production, inappropriate use of antimicrobial drugs, heavy burden of bacterial infectious diseases, huge population without even the rudiments of primary health care, rapid spread through crowding, poor sanitation and sexual contact, self-prescription and easy accessibility of drugs in local pharmacies or open-air markets which is common in most developing countries (Gustafsson and Wide, 1981).

In poorer countries, underuse of drugs encourages resistance. Patients are unable to afford the full course of the drugs or can only afford to buy counterfeit drugs. This exerts a selective pressure that favors the survival of the more resistant microbes. On the other hand, overuse of antimicrobials in food production is also contributing to increased drug resistance. Currently, 50% of all antibiotic production is used to treat sick animals, promote livestock and poultry growth or rid cultivated foods of destructive organisms. In hospitals, inappropriate use of antimicrobial agents tends to create a selective pressure that promotes the emergence of resistant strains and predisposes patients to colonization with such organisms. In many instances, poorly planned or haphazard use of medicine has caused the world to lose these drugs as quickly as scientists have discovered them. Those admitted to hospital wards are especially vulnerable. Globally, 60% of hospital-acquired infections are caused by drug resistant microbes. Globalization, increased travel and trade ensure that resistant strains are quickly spread from their point of origin to other places. A World Health Organization (WHO) report shows that Africa, the Middle East, Latin America and Asia, containing three quarters of the world`s population, have the highest rates of resistance to the older antimicrobial drugs. Antibiotic resistance causes tens of thousands of deaths each year (World Health Organization, 1988).

The aim of the present survey is to investigate the antibiotic susceptibility patterns of bacteria isolates from various parts of the south east and to examine the implications in health and economy.

## MATERIALS AND METHODS

***Test organisms:*** The test organisms included *Escherichia coli* (n=6), *Pseudomonas aeruginosa* (n=9), a typed sample of *Pseudomonas aeruginosa* ATCC 10145, *Salmonella typhi* (n=3), *Klebsiella pneumoniae* (n=1), *Shigella dysenteriae* (n=1), *Staphylococcus aureus* (n=5) and *Streptococcus pneumoniae* (n=1). They were obtained from various sources which included drain water samples from Caritas University, Enugu students' hostels; clinical samples from University of Nigeria Teaching Hospital (UNTH), Utuku-Ozalla, Enugu State, National Orthopaedic Hospital, Enugu, and Federal Medical Centre, Abakaliki, Ebonyi State; Departments of Microbiology of University of Nigeria, Nsukka (UNN), Department of Veterinary Pathology & Microbiology, UNN and Department of biological Sciences, Veritas University, Abuja, Obehie Campus.

Characterization and identification of the samples were carried out according to standard techniques (Cowan and Steel, 1993). The test organisms were stored on Nutrient agar slants in a refrigerator at 4$^{o}$C and sub-cultured every two weeks during the study.

*Standardization of inoculums:* The test organisms were sub-cultured on Nutrient Agar plates and incubated at 37$^{o}$C for 18-24 hours. The growth from each Nutrient Agar plate was transferred into test tubes containing 5ml of 0.9% sterile saline and the volume adjusted in a spectrophotometer to obtain a turbidity which matches that of 0.5 McFarland Standard (containing aproximately$1.5 \times 10^{8}$ cfu/ml).

*Antibiotic discs:*Antibiotic multidiscs obtained from Optun Nig. Ltd and Oxoid Ltd., Basingstoke, Hampshire, England were used. The Gram-negative discs contained ampicillin 30µg, amoxicillin–clavulanic acid (augmentin) 30µg, streptomycin 30µg, gentamicin 10µg, trimethoprim-sulfamethoxazole (septrin) 30µg, ceporex 10µg, ceftriaxone 30µg, nalidixic acid 30µg, ciprofloxacin 10µg, perfloxacin 10µg, ofloxacin 10µg, chloramphenicol 30 µg, nitrofurantoin 300 µg and claritromycin 15µg. The Gram-positive discs

contained ampicillin 30µg, trimethoprim-sulfamethoxazole (septrin) 50µg, clindamycin 10µg, ceftriaxone 30µg, gentamicin 10µg, amoxicillin–clavulanic acid (augmentin) 30µg, erythromycin 10µg, ciprofloxacin 5µg, ofloxacin 5µg, levofloxacin 5µg, norfloxacin 30µg.

*Antibiotic susceptibility testing of the test organisms:*The susceptibilities of the test organisms to the antibiotics were tested according to Kirby-Bauer disk diffusion method. The tests were carried out on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, Hampshire, England). Twenty-five milliliters (25 ml) of sterile molten Mueller-Hinton agar were poured into sterile Petri dishes and allowed to solidify. A sterile cotton swab was immersed in each bacterial suspension. Excess fluid was expressed by rotating the swab against the inside wall of the test tube. This was then used to swab the surface of the Mueller-Hinton agar plates while rotating the plate anticlockwise until the entire surface was swabbed. Within 15 minutes of inoculation, multidiscs containing the antibiotics were placed on the plates inoculated with the test organisms, and allowed to stand for 1 hour for proper diffusion. The plates were then incubated aerobically at an inverted position at 37$^{o}$C for 24 hours. After incubation, diameters of the zones of inhibition (IZD) around each antibiotic disc were measured. IZDs (in millimeters) were recorded by calculating the mean of IZD in replicate plates. The results were interpreted according to Clinical Laboratory Standards Institute (CLSI) Standards (Bauer and Kirby, 1966; CLSI, 2010).

**Method of analysis:** Percentage**s** of resistant, intermediate and susceptible isolates were determined.

## RESULTS AND DISCUSSION

*Results of antibiotic susceptibility testing:* The antibiogram of the test organisms, showing varying degrees of susceptibility is shown in Tables 1 and 2. The following susceptibility profiles were revealed for *E. coli, P. aeruginosa, S. typhi and S. aureus*: *E. coli* (n=6)- resistance 30% to 100%, susceptibility 0%-50%, intermediates 0%-20%; *P. aeruginosa* (n=10)- resistance 30%-100%, susceptibility 0%-60%, intermediates 0%-10%; *S. typhi* (n=3)- resistance 50%-60%, susceptibility 30%-50%, intermediates 0%-10%; *S. aureus* (n=5)- resistance 30%-80.5%, susceptibility 9.75%-50%, intermediates 9.75%-20%. The findings reveal that all the bacteria isolates showed multiple antibiotic resistance ranging from 30% to 100%. A peculiar finding in the study is the occurrence of five 'pan-resistant' isolates which showed resistance (100%) to all the antibiotics tested, representing approximately 18.4% of all the isolates.

The degree of resistance to each antibiotic is taken to be the percentage of isolates resistant to that antibiotic. For Gram-negative isolates, the highest degree of resistance was to ampicillin (100%; 21/21) and nalidixic acid (100%;14/14), followed by trimethoprim-sulfamethoxazole (85.7%;12/14), nitrofurantoin (85.7%;6/7), ceporex (84.6%;11/13), and augmentin (81.0%;17/21). The highest sensitivity was shown to ciprofloxacin (55.4%), followed by perfloxacin (50.0%), streptomycin (46.2%) and clarithromycin (42.9%).

For *S. aureus* (Gram-positive isolate), the highest degree of resistance was to ampicillin (100%;5/5), augmentin (100%;3/3), trimethoprim-sulfamethoxazole (100%;3/3) and cephaplexin (100%;2/2). The highest sensitivity was shown to levofloxaxin (100%), ofloxacin (66.7%) and ciprofloxacin (60.0%).

The data from this investigation suggests that antimicrobial resistance among bacteria pathogens is common and significant in East Nigeria, with many stains of pathogens showing resistance to all tested antibiotics. The findings agree with various reports that resistance is highest in the older, most commonly used antibiotics (Kesah et al. 1999). The high susceptibility to the fluoroquinolones in the study is in consonance with previous findings (Nmema et al., 2009). This supports the claim that ciprofloxacin is still the most effective second line broad spectrum antibiotic in Nigeria, and the only antibiotic currently recommended by WHO for the management of bloody diarrhoea due to resistant *Shigella* organisms globally (Doughari,et al., 2007; World Health Organization, 2013). The explanation for these high resistance rates could be multi-factorial and include underuse or overuse of antibiotics due to poverty and ignorance respectively, self prescription and easy accessibility of drugs in local pharmacies or open-air markets, inappropriate prescription by physicians due to lack of effective antibiotic policies in our hospitals, among other factors (Gustafsson and Wide, 1981). The continuous influx of counterfeit and substandard drugs into the country is an equally worrisome development. Similarly, the propensity of many local manufacturers to produce substandard and counterfeit drugs is well known. The battle to eradicate the local production and importation of sub-standard and fake drugs has been an ongoing program of the National Agency for Food and Drug Administration and

Control (NAFDAC) but this effort is yet to achieve the desired result.

The implications of antibiotic resistance to the health sector are enormous. The alarming rate of resistance in bacteria pathogens raises concern for the effectiveness of antibiotic therapy. This concern is justified by the many cases of treatment failure experienced in antibiotic treatment of infectious diseases. Patients flock to herbalists and quack doctors to seek for alternative treatments which may further compound their health problems. Reports of high morbidity and mortality rates due to antibiotic resistance continue to dominate the pages of health journals (Doughari,et al., 2007;WHO, 1988; WHO, 2013). When infections become resistant to first-line medicines, more expensive therapies must be used. This, coupled with the longer duration of illness and treatment, often in hospitals, increases health-care costs and the economic burden to families and government (WHO, 2013).

The study included bacterial isolates from different sources as well as different areas in the South East as representative organisms. This way the author concludes that multiple antibiotic- resistance was a common feature in all the areas. This seems to lend credence to reports that in sub-Saharan Africa, many strains of bacterial pathogens are now resistant to the repertoire of first-line drugs including ampicillin, chloramphenicol, erythromycin, gentamicin, penicillin, tetracycline and trimethoprim-sulphathoxazole, to which. The available second line antibiotics often include amikacin, amoxicillin-clavulanic acid, cefuroxime, ciprofloxacin and nalidixic acid. Unfortunately, a number of countries do not have a broad enough selection of second-line drugs and so would have difficulty managing resistant infections (Fasehun, 1999; Hart and Kariuki, 1998).

In the present study, the author screened a large number of antibiotics. This has made it possible to see the widespread nature of resistance to all first and second line antibiotics. Worthy of note is the presence of 'pan-resistant' isolates (n=5) which showed resistance to all the tested antibiotics. The emergence of 'pan-resistant' strains in the South East of Nigeria is an indication of the lack of responsibility which has attended the usage of antibiotics in many parts of the country.

The study was undertaken to highlight the antibiotic susceptibility patterns in the area because antibiotic susceptibility patterns directly affect the effectiveness of antibiotic therapy. Many prescribers use antibiotics that are no longer effective due to increased prevalence of resistance, eventually requiring multiple chemotherapeutic courses to effect a cure. This is because surveillance susceptibility testing, which is a valuable tool for cost-effective customization of empiric antibiotic therapy is either lacking or inadequate (Alonge, 2002). The findings of the study will provide needed data that will provoke appropriate action by relevant stakeholders. Future research efforts should be focused on the development of bacteriocins which can replace antibiotics in the treatment of infectious diseases.

*Conclusion* - The findings revealed the alarming and peculiar patterns of antibiotic resistance among bacteria pathogens in the area of study. This highlights the need for appropriate use of antibiotics by health workers and efforts to control procurement and use of antibiotics in the locality which will help to limit the increasing rates of drug resistance in pathogens. Results of this study have important implications for practicing physicians with regard to empirical antibiotic selection. They also have important implications for authorities involved in the development of policies regarding antibiotic utilization, infection control and public healthcare. Our findings call for the implementation of a regional and nationwide surveillance system to monitor antimicrobial resistance trends in Nigeria. Efforts to evaluate the antibiotic-sensitivity pattern of pathogens for commonly used antimicrobial agents in a given locality should be encouraged.

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*Competing interests***:** The author declares that she has no competing interests.

**Table 1:** Susceptibilities of Gram-negative bacteria isolates to antibiotics measured as inhibition zone diameters (mm)

| Test organisms | PN | AU | S | GN | SXT | CEP | CT | NA | CPX | PEF | OFX | C | N | CLR | % S | % I | % R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. coli* 1 | 0 | 0 | 19 s | 19 s | 0 | 0 | - | 0 | 0 | 0 | 0 | - | - | - | 20 | 0 | 80 |
| *E. coli* 2 | 0 | 21s | 30 s | 24 s | 0 | 0 | - | 0 | 29 s | 21 s | 14 i | - | - | - | 50 | 10 | 40 |
| *E. coli* 3 | 0 | 0 | - | 0 | - | - | 0 | - | 0 | 10 r | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| *E. coli* 4 | 0 | 0 | - | 14 i | - | - | 28 s | - | 20 i | 20 s | 0 | 20 s | 20 s | 20 s | 50 | 20 | 30 |
| *E. coli* 5 | 0 | 0 | - | 0 | - | - | 0 | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| *E. coli* 6 | 0 | 0 | - | 19 s | 0 | - | - | 0 | 0 | - | 22 s | - | - | - | 28.7 | 0 | 71.3 |
| *P. aeruginosa* 1 | 0 | 0 | 0 | 22 s | 14 r | 24 s | - | 0 | 36 s | 28 s | 24 s | - | - | - | 50 | 0 | 50 |
| *P. aeruginosa* 2 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 18 i | 0 | 0 | - | - | - | 0 | 10 | 90 |
| *P. aeruginosa* 3 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 24 s | 0 | 0 | - | - | - | 10 | 0 | 90 |
| *P. aeruginosa* 4 | 0 | 0 | 22 s | 12 r | 13 i | 0 | - | 0 | 34 s | 30 s | 24 s | - | - | - | 40 | 10 | 50 |
| *P. aeruginosa* 5 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | - | - | - | 0 | 0 | 100 |
| *P. aeruginosa* 6 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 22 s | 0 | 0 | - | - | - | 10 | 0 | 90 |
| *P. aeruginosa* 7 | 0 | 0 | 22 s | 12 r | 13 r | 0 | - | 0 | 34 s | 30 s | 24 s | - | - | - | 40 | 0 | 60 |
| *P. aeruginosa* 8 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | - | - | - | 0 | 0 | 100 |
| *P. aeruginosa* 9* | 0 | 0 | 21 s | 15 s | 15 i | 21 s | - | 0 | 32 s | 29 s | 21 s | - | - | - | 60 | 10 | 30 |
| *P. aeruginosa* 10 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | - | - | - | 0 | 0 | 100 |
| *S. typhi* 1 | 0 | 18 s | 22 s | 16 s | 0 | 0 | - | 0 | 28 s | 0 | 0 | - | - | - | 40 | 0 | 60 |
| *S. typhi* 2 | 0 | 14 i | - | 0 | - | - | 0 | - | 22 s | 19 s | 10 r | 19 s | 0 | 0 | 30 | 10 | 60 |
| *S. typhi* 3 | 12 r | 0 | - | 16 s | - | - | 15 r | - | 26 s | >28 s | 20 s | 0 | 0 | 14 s | 50 | 0 | 50 |
| *K. pneumonia* | 13 r | 16 i | - | 16 s | - | - | 22 i | - | 22 s | >28 s | 24 s | 0 | 11 r | 18 s | 50 | 20 | 30 |
| *S. dysenteriae* | 9 r | 9 r | - | 7 r | - | - | 18 r | - | 20 i | 21 s | 9 r | 10 r | 10 r | 9 r | 10 | 10 | 80 |
| **Degree of Resistance (%)** | 100 | 81.0 | 53.9 | 57.1. | 85.7 | 84.6 | 71.4 | 100 | 33.3 | 50.0 | 61.9 | 71.4 | 85.7 | 57.1 | | | |

PN- Ampicillin, AU-Amoxicillin-clavulanic acid (Augmentin), S- Streptomycin, GN- Gentamicin, SXT- Trimethoprim-Sulfamethoxazole(Co-trimoxazole or Septrin), CEP-Ceporex, CT-Ceftriaxone, NA- Nalidixic acid, CPX-Ciprofloxacin, PEF-Perfloxacin, OFX-Ofloxacin, C-Chloramphenicol, N- Nitrofurantoin, CLR-Clarithromycin, * ATCC Typed Sample, - Not tested, s –susceptible, i –intermediate, r –resistant.

**Table 2:** Susceptibilities of Gram-positive bacteria isolates to antibiotics measured as inhibition zone diameters (mm)

| Test organisms | PN | AU | GN | SXT | CE | CT | CPX | OFX | LV | NOR | CD | E | % S | % I | % R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *S. aureus* 1 | 0 | 0 | 12 r | 0 | - | - | 32 s | 28 s | - | - | 0 | 26 s | 37.5 | 0 | 62.5 |
| *S. aureus* 2 | 0 | - | 0 | - | 10 r | 0 | 16 i | 9 r | 19 s | 0 | 0 | 8 r | 10 | 10 | 80 |
| *S. aureus* 3 | 0 | - | 18 s | - | 10 r | 12 r | 28 s | 28 s | 30 s | 14 i | 28 s | 18 i | 50 | 20 | 30 |
| *S. aureus* 4 | 0 | 0 | 20 s | 0 | - | 0 | 0 | - | - | - | - | 19 i | 14.35 | 14.35 | 71.3 |
| *S. aureus* 5 | 0 | 0 | 14 i | 0 | - | 0 | 0 | - | - | - | - | 8 r | 9.75 | 9.75 | 80.5 |
| *S. pneumonia* | 0 | - | 8 r | - | 0 | 8 r | 18 i | 18 s | 22 s | 0 | 0 | 0 | 20 | 10 | 70 |
| **Degree of Resistance (%)** | 100 | 100 | 40.0 | 100 | 100 | 100 | 40.0 | 33.3 | 0.00 | 50.0 | 66.7 | 40.0 | | | |

PN-Ampicillin, SXT- Trimethoprim-Sulfamethoxazole(Co-trimoxazole or Septrin), CD-Clindamycin, CE-Cephaplexin, CT-Ceftriaxone, GN-Gentamicin, AU-Amoxicillin-clavulanic acid (Augmentin), E-Erythromycin, CPX-Ciprofloxacin, OFX-Ofloxacin, LV-Levofloxacin, NOR-Norfloxacin, - not tested, s –susceptible, i –intermediate, r –resistant.

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