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### Antibiotic Susceptibility Profile of Bacteria spp. Isolated from Patients with Recurrent Cough in Cross River State, Nigeria

\*Akpan, M. M., Itah, A. Y., Akinjogunla, O. J. and Eshiet, U. M.

Department of Microbiology, University of Uyo, Uyo, Akwa Ibom State, Nigeria

#### ABSTRACT

A study on bacterial species associated with recurrent cough was carried out using Standard Microbiological Techniques [SMT] and their susceptibility to some commonly available antibiotics was evaluated using Disc Diffusion method [DDM]. The prevalence of bacteria spp. isolated were as follows: *Streptococcus pneumonia* [20.4%], *Streptococcus pyogenes* [12.5%], *Staphylococcus aureus* [18.5%], *Klebsiella pneumonia* [20.5%], *Escherichia coli* [7.7%], *Pseudomonas aeruginosa* [6.6%], *Haemophilus influenza* [0.1%] and *Corynebacterium diphtheria* [13.7%]. The susceptibility patterns varied from one bacterial isolates to the other depending on the drug used. *Streptococcus pneumoniae* was 100% resistant to chloramphenicol, 95.6% resistant to cloxacillin and 84.8% resistant to tetracycline. *Haemophilus influenzae* was 100% resistant to ampicillin, erythromycin, cloxacillin and cotrimoxazole. 50% of the bacterial spp were resistant to the other antibiotics used except flouroquinolones. *Klebsiella pneumoniae* was 100% resistant to both tetracycline and chloramphenicol. Some of the bacteria exhibited multiple antibiotics resistance (MAR). However, this results show that the most efficacious antibiotics for the treatment of patients with recurrent cough were sparfloxacin and ciprofloxacin due to the high level of some resistance of the bacterial spp. associated with recurrent cough to other commercial sold drugs.

#### INTRODUCTION

Cough is a sudden, often repetitive spasmodic contraction of the thoracic cavity resulting in violent release of air from the lungs and usually accompanied by distinctive sound [1]. Coughing is an action the body takes to get rid of substances that are irritating the air passages. A cough is usually initiated to clear a build up of phlegm in the trachea. Air may move through the passages at up to 480km/h [300mph] during contraction [2]. Cough can be dry or productive. A productive cough is one that brings up mucus while a dry cough is one that does not bring up mucus [2].

The mucocilliary blanket of the respiratory epithelium traps microorganisms less than 10µm in diameters that are deposited on the mucosal surface and transport them by ciliary action away from the lungs. The microorganisms larger than 10µm usually are trapped by hair and cilia lining

the nasal cavity. Coughing and sneezing reflexes clear the respiratory system of microorganisms by expelling them forcefully from the lungs through the mouth and nose respectively [3 ].

Cough is caused by organisms such as *Haemophilus influenza*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*,  $\beta$ -hemolytic *Streptococci*, *Pneumococci* and the aerobic *cocci*. *Pseudomonas aeruginosa* causes a particularly severe and interactable bronchopneumonia. *Staphylococcus aureus* is one of the pathogens associated with chronic and acute cough infections. It is implicated in recurrent cough, much more than any other organism. The organism is present in the respiratory tract in variable amounts in healthy persons as an opportunistic pathogen [4 ]. Antibiotics are used to treat many infectious diseases. Antibiotics inhibit or kill the pathogen without causing much harm on the host [5 ]. Natural antibiotics have been found that can damage pathogens in diverse ways. The mechanisms of action of antibiotics include: Inhibition of transpeptidation, inhibition of protein, folic acid synthesis, inhibition of bacterial DNA-dependent RNA *polymerase* among others [6 ].

In the 21st century, one of the most serious threats to public health is the spread of drug resistant pathogens [7 ]. Bacteria are resistant to antibiotics in different ways. [8 ] stated that antibiotics used and misused contribute to the spread of drug resistance. Bacteria also develop mutations in structural genes for drug targets [9]. Bacteria may display antibiotic resistance due to the following: lack of target for antibiotic; antibiotic target may be inaccessible; antibiotic target may be modified to prevent the action of the drugs; the antibiotic may be chemically modified or destroyed; bacteria may elaborate alternative pathways avoiding the drug target, and antibiotics may also be enzymatically degraded to an inactive form [5 ;9]. The aims of this work were to determine bacterial spp. associated with recurrent cough in this region and their antimicrobial susceptibility patterns.

## MATERIALS AND METHODS

### Sources and Collection of Samples

Sputa were aseptically collected in sterile bottles from in-patients and out-patients with recurrent cough in some selected hospitals in Cross River State. Which were: General Hospital, Calabar [GHC]; Infectious Disease Hospital, Ogoja [IDHO]; General Hospital, Ugep [GHU]; General Hospital, Ikom [GHI]; General Hospital Obudu [GHB]; General Hospital, Ogoja [GHO] and General Hospital, Akamkpa [GHA].

### Processing of Samples for Microscopic Examination

Each sputum collected from the patients with recurrent cough was identified preliminarily for the presence of Acid-fast bacilli using Ziehl Neelsen's staining techniques as described by [10-12 ].

### Sputum Culture and Identification of Bacteria

A loopful of each sputum sample was aseptically cultured on MacConkey agar, Blood agar, and Chocolate agar for microbial morphology and identification. The cultures on blood agar and MacConkey agar were incubated aerobically at 37<sup>0</sup>C for 24 hours. While the cultures on chocolate agar were incubated in carbon [IV] oxide enriched atmosphere by placing a candle light in the enclosed container [tin] along with the culture plates and incubated at 37<sup>0</sup>C for 24 hours. All the isolates were characterized and identified by microscopic examination, colonial morphology and biochemical tests. The biochemical tests carried out included Gram reaction, catalase, coagulase, oxidase, citrate, urease, bile solubility, indole, Voges-Proskauer, methyl red, nitrate reduction, motility, sugar fermentation profile and the presence or absence of endospore [13;14 ].

**Antimicrobial susceptibility Test**

The antibiotic susceptibility test was determined by the standardized disc diffusion method: Antibiotics used include Tetracycline [Tet] [25µg]; Ampicillin [Amp] [25µg]; Penicillin [Pen] [25µg]; Erythromycin [Ery] [5µg]; Cloxacillin [Cxc] [5µg]; Gentamycin [Gen] [19µg]; Cotrimoxazole [Cot] [25ml]; Chloramphenicol [Chl] [30µg]; Sparfloxacin [Dc] [10µg]; Ofloxacin [Ofl] [5µg]; Perflacin [Pef] [5µg]; Ciprofloxacin [Cip] [30µg] and Streptomycin [Str] [30µg]. Sterile peptone broth was aseptically inoculated with the test organism. The culture was incubated for 6hrs. The spread plate technique was used to inoculate the bacterial suspension on Mueller-Hinton agar. The surface of the plate was allowed to dry for about 5 minutes, then sterile forceps was used to pick the antibiotic discs and placed gently and firmly on the surface of the seeded plate. The discs were evenly distributed to allow an edge not less than 20mm from the wall of the Petri dish. The inoculated plates were incubated at 37<sup>0</sup>C for 24 hours. Zones of inhibition after 24 hrs were observed and the diameters of inhibition zones were measured in millimeter using a ruler. The interpretation of the measurement as sensitivity, intermediate and resistant was made according to manufacturer's standard zone size interpretative manual. The intermediate readings were considered as sensitive for the assessment of the data.

**RESULTS AND DISCUSSION**

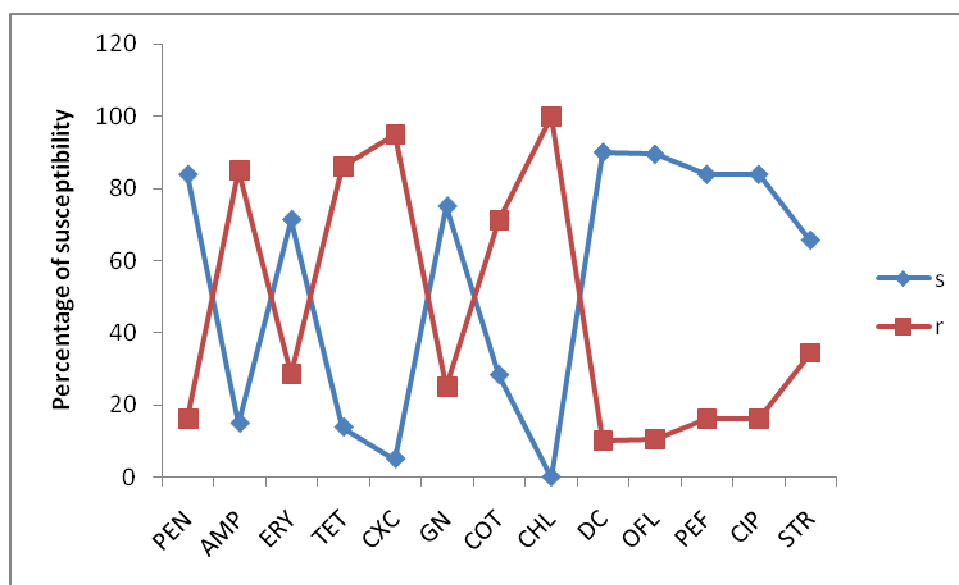
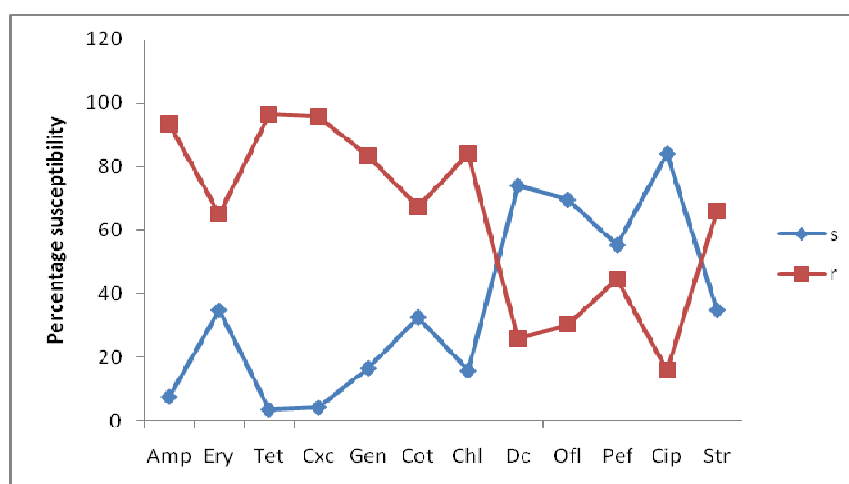
A total of 1372 specimens were collected for studies; the highest number of specimens were obtained from IDHO while the least was obtained from GHA [Table 1]. The following bacterial spp. were isolated. *Streptococcus pneumonia* 296[20.4%] *Staphylococcus aureus* 269[18.5%] *Haemophilus influenzae*,2[0.1%] *Streptococcus pyogenes*,182[12.5%] *Klebsiella pneumoniae* 298[20.5] *Corynebacterium diphtheriae*,199[13.7%] *Pseudomonas aeruginosa* 96[6.6%] and *Escherichia coli* 112[7.7%] [Table 2]. This proves that the recurrent cough is due to infections of the respiratory tract by certain microorganism. The degree of resistance of the bacterial isolates to antimicrobial agents varied from one drug to another and from organism to organism. All the *Streptococcus pneumoniae* isolated were resistant to chloramphenicol followed by cloxacillin, tetracycline, ampicillin and cotrimoxazole [Fig1]. All the *Haemophilus influenzae* isolated from the different Hospitals were resistant to erythromycin, tetracycline, cloxacillin, cotrimoxazole and ampicillin [Fig3]. All *Klebsiella pneumoniae* exhibited high resistant to tetracycline and chloramphenicol [Fig4]. The degree of resistance of *S. aureus* to ampicillin, cloxacillin, tetracycline and gentamicin was on a high side [Fig2]. Other isolates exhibited multiple drug resistance patterns [Figs 5-8].

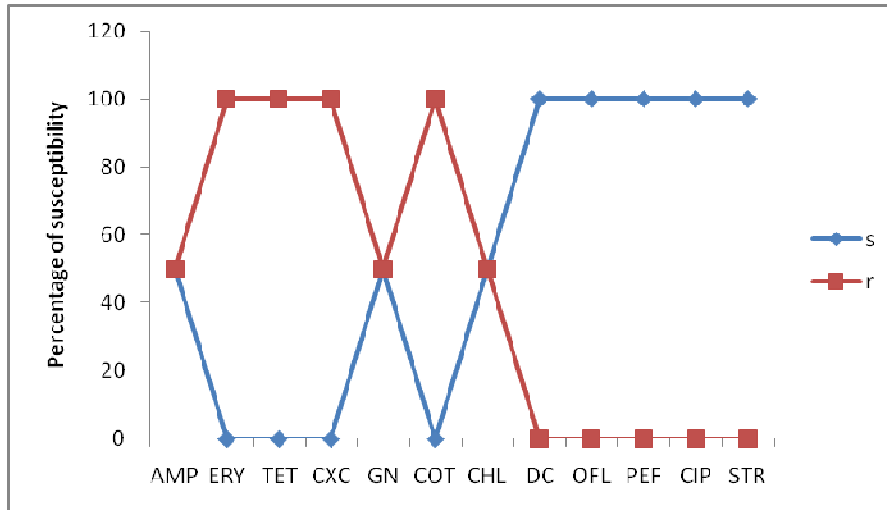
**Table1: Number of samples collected from different locations**

Locations	Number of samples
IDHO	532
GHC	158
GHO	110
GHU	122
GHI	218
GHB	176
GHA	56
TOTAL	1372

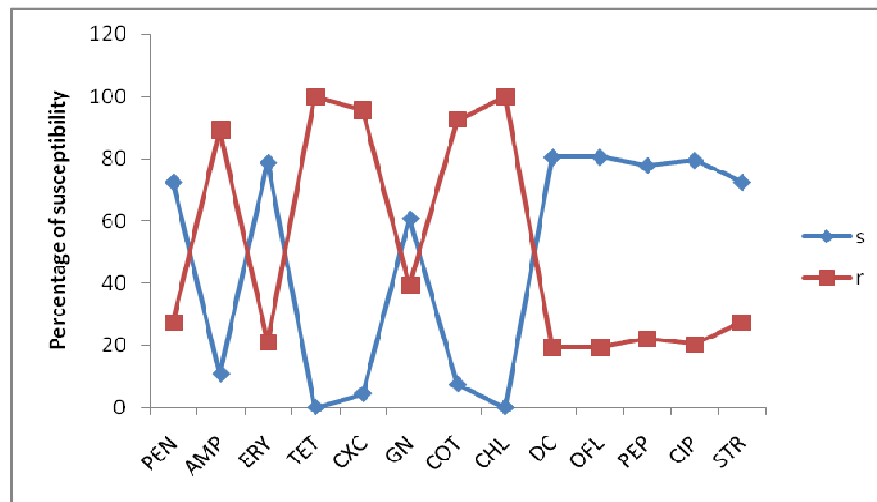
Table 2: Bacteria spp Isolated from Sputum of Patients with Recurrent Cough in Cross River State

Bacteria isolated	No. of Occurrence	% of Occurrence
<i>Streptococcus pneumonia</i>	296	20.4
<i>Staphylococcus aureus</i>	269	18.5
<i>Klebsiella pneumonia</i>	298	20.5
<i>Escherichia coli</i>	112	7.7
<i>Streptococcus pyogenes</i>	182	12.5
<i>Pseudomonas aeruginosa</i>	96	6.6
<i>Haemophilus influenza</i>	2	0.1
<i>Corynebacterium diphtheria</i>	199	13.7
Total	1454	100

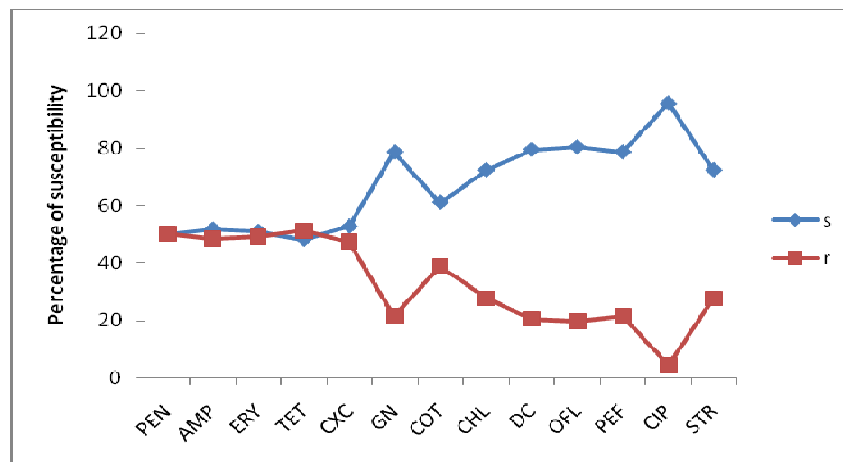
Fig 1: Susceptibility Patterns of *Streptococcus pneumoniae* Isolated from Patients with Recurrent Cough. S= Sensitive, R=ResistanceFig2: Susceptibility Patterns of *S. aureus* Isolated from Patients with Recurrent Cough. S= Sensitive, R=Resistance



**Fig3: Susceptibility Patterns of *Haemophilus influenzae* Isolated from Patients with Recurrent Cough.**  
*S= Sensitive, R=Resistance*



**Fig4: Susceptibility Patterns of *K. pneumoniae* Isolated from Patients with Recurrent Cough.**  
*S= Sensitive, R=Resistance*



**Fig5: Susceptibility Patterns of *E. coli* Isolated from Patients with Recurrent Cough.**  
*S= Sensitive, R=Resistance*

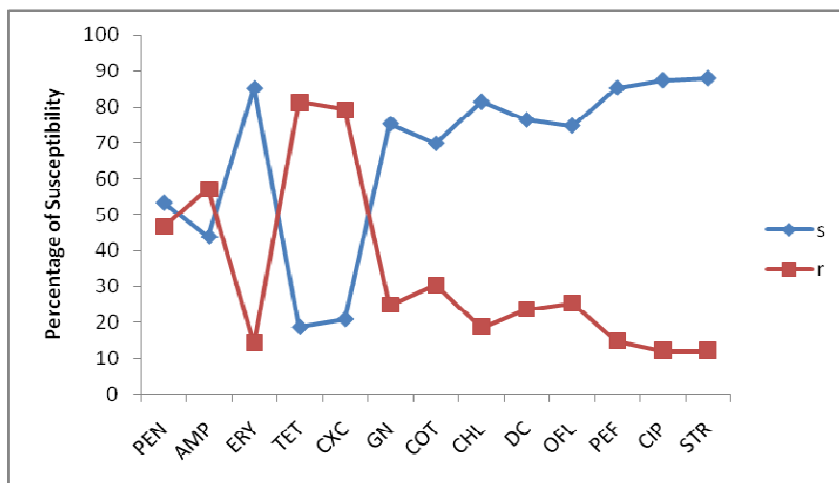


Fig6: Susceptibility Patterns of *S. pyogenes* Isolated from Patient with Recurrent Cough. S= Sensitive, R=Resistance

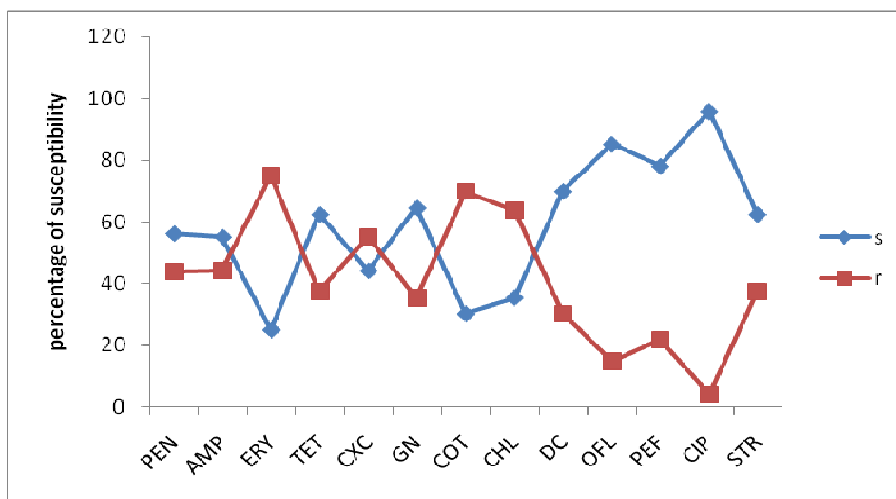


Fig7: Susceptibility Patterns of *P. aeruginosa* Isolated from Patients with Recurrent Cough S= Sensitive, R=Resistance

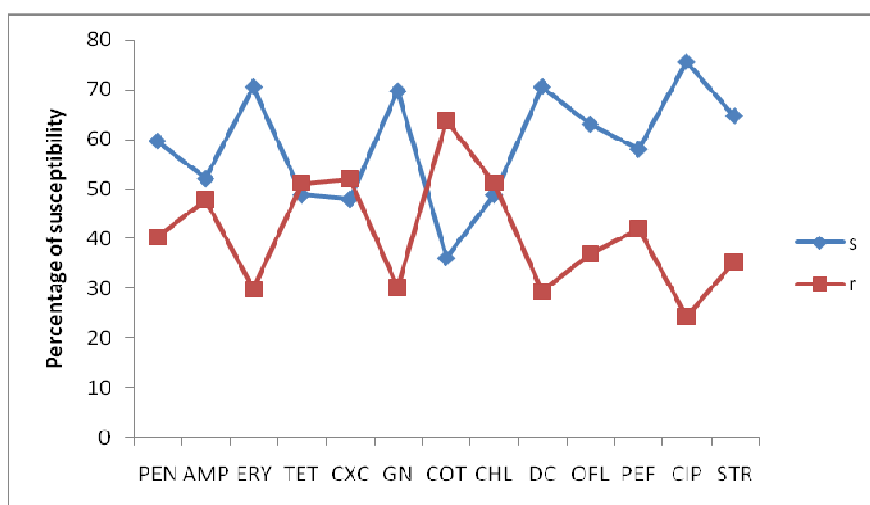


Fig8: Susceptibility Patterns of *C. diptheriae* Isolated from Patients with Recurrent Cough. S= Sensitive, R=Resistance

The results obtained from this study revealed the presence of bacteria in sputa of patients with recurrent cough. The earlier investigators [7;15;16;17]. documented the health risks associated with *Escherichia coli* and *Staphylococcus aureus*. The clinical bacteria isolated from all sputum samples of recurrent cough patients exhibited multiple resistance to antibiotics. This agrees with the report of [5;6;17;18;19]. The reason for the resistance may be due to indiscriminate use and abuse of drugs, adulteration of drugs and mutation of microorganisms. Most of the clinical bacterial isolates exhibit high resistant to ampicillin, tetracycline, cloxacillin, cotrimoxazole, chloramphenicol, antibiotics. For these group of antibiotics to be used for treatment of recurrent cough sensitivity test should be conducted. Health sectors should educate public on proper usage of antimicrobial agents.

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