Int. J. Pure Appl. Sci. Technol., 10(2) (2012), pp. 62-70

International Journal of Pure and Applied Sciences and Technology

ISSN 2229 - 6107

Available online at ***www.ijopaasat.in***

Research Paper

Effects of Antibiotic Self-Medication on the Efficacy of Four Antibiotics Commonly used in Ghana on Clinically Isolated Micro Organisms

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(Received: 21-5-12; Accepted: 13-6-12)

Abstract: *Antibiotic resistance is associated with antibiotic abuse through self medication. Antibiotic resistance is established through antimicrobic susceptibility testing of isolated pathogens to the antibiotic of interest. Between June and October 2008, 150 urine samples collected from patients referred to a Clinical Laboratory in Accra Ghana for clinical laboratory tests were processed. Positive and negative cultures were 60% and 40% respectively of all samples. 34.7% of patients had self medicated and 65.3% had not. 54.24% of the negative cultures were from the self medicated subjects and 45.76% from non-self medicated subjects. 78.02% of positive cultures were from non-self medicating subjects while 21.98% were from self medicated subjects. E. coli, S. aureus and Klebsiella pneumonia were isolated according to standard methods and their sensitivities to Augmentin, Gentamycin, Imipenem and Amoxicillin antibiotics determined by Kirby-Bauer disk diffusion method. K. pneumoniae was totally resistant to Gentamycin, Augmentin and Amoxicillin; E. coli was totally resistant to Amoxicillin and S. aureus totally sensitive to Imipenem. Generally, more antibiotic self-medicating individuals had resistant pathogens than non self-medicating individuals. The results confirm that antibiotic self medication prior to clinically prescribed antibiotic treatment has a significant influence on the response of bacteria to the clinically administered antibiotics.*

Keywords: Antibiotics, Self-medication, Pathogens, Resistant, Sensitive.

Introduction

Antibiotics are antimicrobial agents or chemicals produced by microorganisms to kill or to inhibit the activities of other microorganisms. For an antibiotic to be useful, it must be effective but must also be selective in its toxicity, killing infectious agents but not patients (Todar, 2003). Bactericidal

antibiotics kill bacterial while bacteriostatic antibiotics only halt the growth of bacterial but become bactericidal at high concentrations (Stuart, 1998).
Antibiotics are effective in three different ranges. Broad spectrum antibiotics (e.g. Amoxicillin, Streptomycin, Tetracycline, and Chloramphenicol) are effective against a wide range of both gram-positive and gram-negative bacteria. Narrow spectrum ones (e.g. Penicillin G, Gentamycin) are effective against either gram-positive or gram negative bacteria or only a few specified species. Limited spectrum antibiotics are effective only against a single organism or disease.
The modes of action of antibiotics are: Interference with Cell Wall Synthesis (beta-lactam antibiotics penicillin and cephalosporin); Interference with Cell Membrane Function (Polymyxim B); Interference with Protein Synthesis (Streptomycin, Tetracycline, Chloramphenicol and Erythromycin); Interference with Nucleic Acid Synthesis (Rifampin and Nalidixic acids antibiotics); Interference with Metabolic Activity (sulfa antibiotics) (Krasner, 2002).
When antibiotics are misused or inappropriately applied, some variant bacteria may survive the treatment. The antibiotic does not technically cause the resistance, but allows it to happen by creating a situation where "normal" bacteria are eliminated from the system thereby allowing room for an already existing variant to flourish.
The four main mechanisms by which microorganisms exhibit resistance to antimicrobials are by: drug inactivation or modification by Beta-lactamase enzymes produced by some bacteria which break the beta-lactam ring of Beta-lactam antibiotics like Penicillin's, Cephalosporin's, Cephamycins; Inhibition by of the synthiesis of Peptidoglycan, which is the major component of bacterial cell walls leads to irregularities in cell wall structure such as elongation, lesions, loss of selective permeability, and eventual cell death and lysis; Alteration of metabolic pathways; and reducing drug accumulation in the cell by decreasing drug permeability or increasing active pumping out of the drugs from the cell.
Helegbe *et al* (2009) screened some commonly used antibiotics in Ghana for their efficacy in treating diseases. The disc susceptibility test was used to screen stock antibiotics such as Ampicilline, Chloramphenicol, Kanamycin and Penicillin based antibiotics from different manufacturers (both local and foreign) which were obtained from different pharmacy shops against some bacteria species such as *Salmonella typhi*, *Staphyloccus aureus* and six strains of *E. coli*. The study showed that both stock and field antibiotics (Antibiotics obtained from pharmacy shops), J916 (an *E. coli* isolate) and *Salmonella typhi* were found to be less sensitive to the penicillin-based antibiotics and both locally and foreign manufactured antibiotics appeared to be effective against the select bacteria. According to Adu – Sarkodie (1997) self medication of antibiotics is especially wide spread in developing countries. The antimicrobial self medication practices of 764 patients attending an STD clinic in a developing country were studied. Seventy-four and a half per cent admitted to self medication before reporting to the clinic. Such antibiotics are sold over the counter by both trained and untrained personnel, given by friends or were 'left-over's' from previous medications and are taken in inappropriate dosages and could cause antibiotic resistance in patients.
The objectives of this study were to determine the prevalence of self-medication among patients presenting at a clinical diagnostic laboratory prior to clinical prescription of antibiotic treatment, and to determine the effects of antibiotic self-medication on the responses of three clinically isolated pathogens to four commonly used antibiotics in Ghana.

Materials and Methods
Outline of Procedure

The subjects were one hundred and fifty patients of all age groups who had been referred from various hospitals to a Clinical Laboratory in Accra Ghana for clinical laboratory tests prior to the administration of clinically approved antibiotic therapy by the hospitals. Patients who were found to be on clinically prescribed antibiotics as indicated by the physician were excluded from the research. Patients were interviewed and then grouped into two: those who had self medicated before reporting to the hospital for treatment and those who had not. The groupings were confirmed by testing the urine of each subject for antimicrobial activity after which the urine samples were also grouped into two: Samples which reported positive for antimicrobial activity and samples which reported negative

for antimicrobial activity. All the urine samples from in both groups were cultured to identify types of bacteria in them. Susceptibility tests of the isolated pathogens from the two groups of samples were done against four antibiotics, i.e., Augmentin, Gentamycin, Imipenem and Amoxicillin. Zones of inhibition were measured and compared against a standard chart to determine the sensitivities of the isolated pathogens to the antibiotics.

Collection of Urine Samples

Urine samples were collected in sterile plastic containers on which the date, name, age, sex, and laboratory number of each patient was inscribed. The appearance of the urine was observed and recorded as cloudy, semi-cloudy or clear. Samples were processed within two (2) hours of collection. Each sample was taken through microscopy for examination of the wet mount, bacterial culture and biochemical tests, and antibiotic susceptibility tests.

Testing for Antimicrobial Substances in Urine as Confirmation of Self Medication

E. coli was used as the test organism. *E. coli* is sensitive to Augmentin, Gentamycin and Imipenem and resistant to Amoxicillin. This organism was chosen due to its sensitivity to a wide range of antibiotics available (that are likely to have been used or abused by the subject) and its resistance to only a few available antibiotics. It is therefore more likely to have its growth inhibited by a significant range of antibiotics that are likely to be present in the urine of the subjects.
An entire Mueller Hinton agar plate was streaked with *E. coli* suspension. The plate was divided into eight parts. A paper strip dipped into the urine sample was inoculated unto a portion of the divided plate. The inoculated plates were incubated for 18 hours at 35°C. A zone of inhibition of any size suggested the presence of antimicrobial substance in the urine sample. The report was given as "Antimicrobial Substance" present or absent.

Microscopic Examination of Wet Amount

A wet preparation was done on each sample and observed under the microscope using x40 magnification. The wet preparation was used to examine for the presence of bacteria, white blood cells, red blood cells, epithelial cells and yeast cells.

Preliminary Tests for the Identification of Microbes

A sterilized calibrated loop was used to take a loopful of unspan (not centrifuged) but well mixed urine which was then inoculated onto Uriselect agar and streaked out for single colonies. Inoculated plates were incubated for 18 hours at 35^{o}C and then examined to identify the microbes.

Colonial Morphology

Colonies were examined under the microscope for their morphologies and this was the preliminary identification made before confirmatory biochemical tests were carried out to ascertain the validity of predictions of the strains of bacteria.

Gram Staining

Gram Staining with microscopic observation was done under oil-immersion objective (X100). Gram positive bacteria appeared dark purple. Gram negative bacteria appeared pale to dark red.

Confirmatory Biochemical Tests for the Identification of Microbes

Catalase Test
This test differentiates between bacteria that produce Catalase (*Staphylococci* species) from non-Catalase producing bacteria (*Streptococcus* species). A positive test indicated the presence of *Staphylococci* species.

Coagulase Test
This test differentiates between *S. aureus* and other Staphylococcus species. A positive test indicated the presence of *S. aureus*.

Citrate Utilization Test
Koser's citrate medium inoculated with the test organism was used to test for *Klebsiella species*. Turbidity and a blue colour of the test medium showed the presence of *Klebsiella species*.

Indole Test
Kovacs' reagent was added to the microbial culture in tryptone broth and incubated at 35°C - 37°C overnight. A red layer at the top of the culture indicated the presence of *E. coli*.

Standard Strains for Quality Control
Standard reference strains of bacteria were tested in parallel with the clinical cultures. *S. aureus* (NCTC 6571) – For Gram positive bacteria and *E. coli* (NCTC 10418) – For Gram negative bacteria and *Pseudomonas aeruginosa* (NCTC 10622) – for *Pseudomonas aeruginosa* were used.

Antimicrobic Sensitivity Tests
The test was done for organisms previously isolated from the Uriselect agar using the Kirby-Bauer method (Benson, 2001). Ampicillin, Gentamycin, Amoxicilin and Imipenem were purchased from various pharmacy shops in Accra. Samples were checked for their batch numbers, manufacturer's origin and date of expiry. The antibiotics were dissolved in sterilized distilled water to make a stock concentration of 50 μg/20 μL^{-1}. A few drops of 25% NH_4OH was added to dissolve the antibiotic and filtered through 200 nm pore size membrane filter for sterilisation.

Inoculum Preparation
3 – 5 well isolated colonies of the same morphological type were selected from an agar plate culture. Each colony was picked with a sterile wire loop and transferred into a test tube containing 2mls of Tryptic Soy Broth (TSB) to form a suspension. When the turbidity of the suspension was similar to that of the 0.5 McFarland turbidity standards, no further incubation was needed. If the turbidity was lower than the standard, it was incubated at 35°C until it achieved the turbidity of the standard. When needed sterile saline was added to achieve the turbidity of the standard.

Preparation of Mueller Hinton Agar Test Plates
Within 15 minutes after the inoculum suspension achieved the turbidity standard a sterile non-toxic cotton swab on an applicator was dipped into the culture suspension in a tube, excess suspension expressed by rotating the tube several times whiles pressing the swab firmly on the inside wall of the tube above the fluid level and then used to swab the entire dry surface of a Mueller Hinton agar. The agar plate was left for 3 – 5 minute to allow for any excess surface moisture to be absorbed before applying the antibiotic discs. Separate plates were prepared for *E. coli, S. aureus* and *K. pneumoniae*.

Preparation and Impregnation of Antimicrobial Discs
A perforator was used to punch several discs of 6.0 mm diameter from Whatman filter No. 1 paper The discs were sterilized in a Petri dish in an oven at 160.0°C for 15 min. Randomly selected discs were placed on previously sterilised Mueller Hinton Agar plates and incubated at 37°C overnight. Sterilized discs were selected and allowed to cool. Discs were impregnated separately with 30 μg of

Augmentin, 10 µg Gentamycin, 30 µg Imipenem and 25 µg Amoxicillin respectively and dried in Petri dishes in an incubator at 37°C.

Measurement of Zones of Inhibition

The various antibiotic discs were picked with sterile forceps and placed at uniform distances apart on the surfaces of the Mueller Hinton agar plates of the various pathogens. The discs were pressed down gently to ensure complete contact with the agar surfaces. Within 15 minutes after discs were applied the plates were inverted and placed in an incubator set to 35°C; for 18 hours. The plates were examined and the diameters of the zone of inhibition measured to the nearest millimeter and compared to that on a standard table in order to classify the microorganism as Resistant (R), Intermediate (I) or Sensitive (S) to the antibiotic.

Results and Discussions

Sixty percent of the subjects had pathogens in their urine **(Fig 1).**

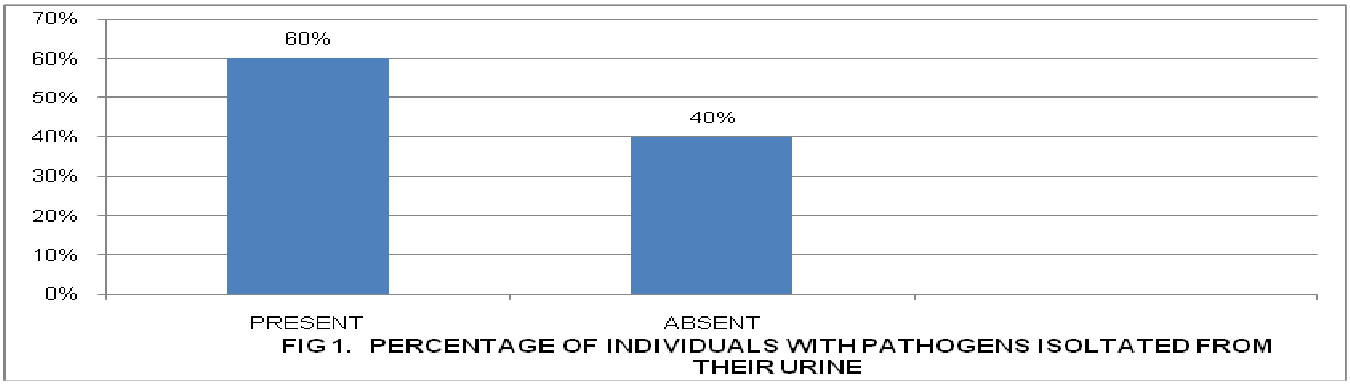

FIG 1. PERCENTAGE OF INDIVIDUALS WITH PATHOGENS ISOLTATED FROM THEIR URINE

Urine samples from 34.7% of the subjects tested positive for antibiotics while 65.3% of the subjects tested negative (Fig 2). The observation confirms the practice of self medication of antibiotics among patients prior to reporting to health facilities and agrees with the works of Ebor *et al* , (1987) and Chrétien *et al* , (1975) that people who reported at clinics for various infections had self-medicated with antibiotics before seeking medical treatment from a physician.

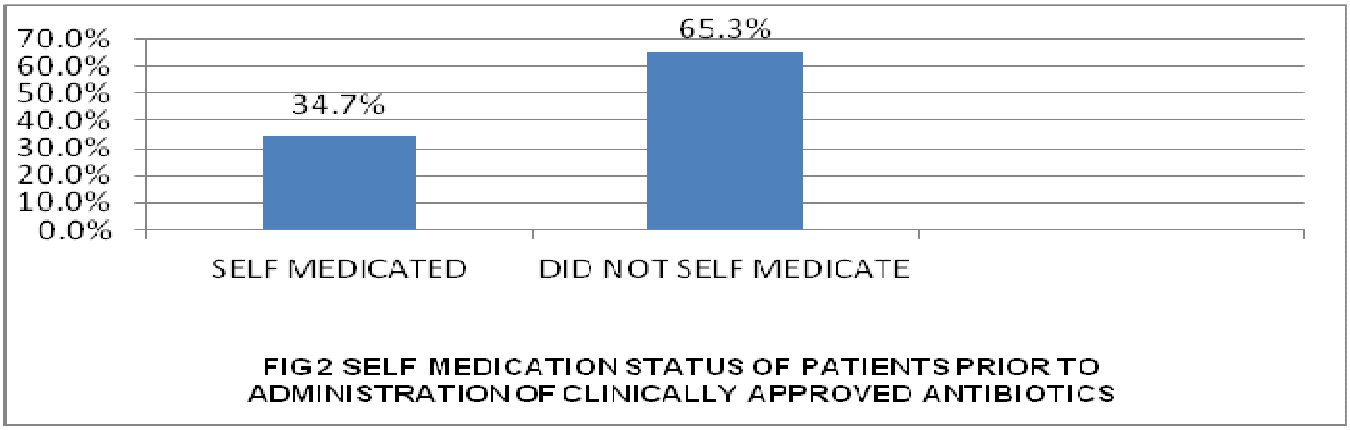

FIG 2 SELF MEDICATION STATUS OF PATIENTS PRIOR TO ADMINISTRATION OF CLINICALLY APPROVED ANTIBIOTICS

E. coli were present in eighty percent of the pathogen-contaminated samples, *S. aureus* in twelve percent and *K. pneumoniae* in eight percent (Fig 3). The results show that *E. coli* was the pathogen found in most of the subjects and this confirms the observation by the National Kidney and Urologic Diseases Information Clearinghouse (NKUDIC) of the US Department of Health and Human Services (2010) that most urinary tract infections are caused by *E. coli*.

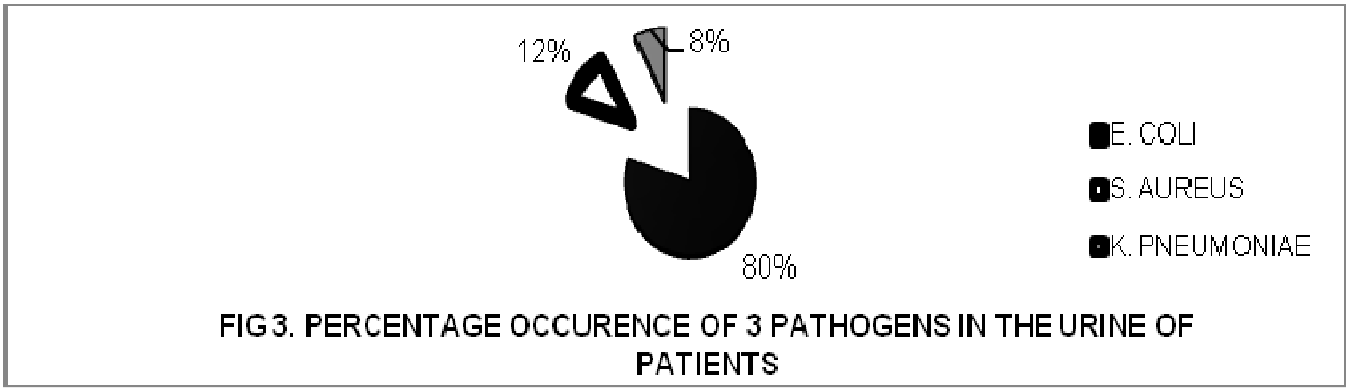

FIG 3. PERCENTAGE OCCURENCE OF 3 PATHOGENS IN THE URINE OF PATIENTS

For urine samples from patients who self medicated with antibiotics 21.98% contained pathogens while 54.24% did not. For urine samples from patients who did not self medicate 78.02% contained pathogens while 45.76% did not (Fig 4). This shows that self medication significantly inhibits the growth of pathogens in urine.

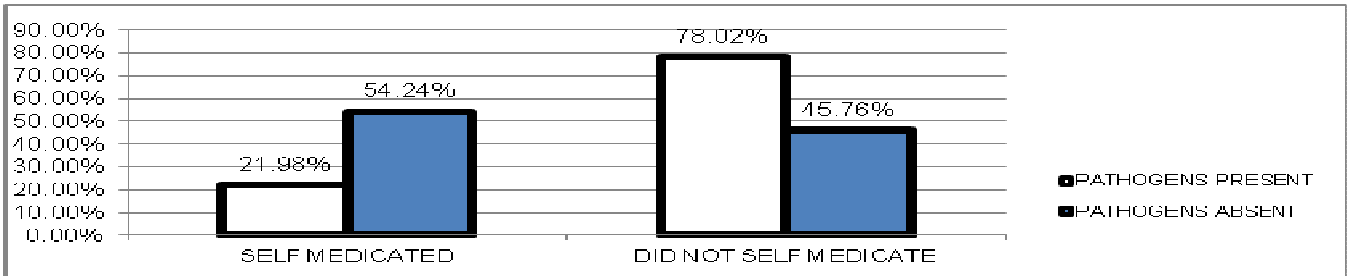

FIG 4. SELF MEDICATION STATUS OF PATIENTS PRIOR TO ADMINISTRATION OF CLINCALLY APPROVED ANTIBIOTICS AND THE OCCURENCE OF PATHOGENS IN THEIR URINE

From the sensitivity tests *K. pneumoniae* showed total resistance to Gentamycin, Augmentin and Amoxicillin (Fig 5, 6 and 8). Akortha *et al* (2010) reported that Amoxicillin resistance gene (amxr) was detected in 88.1% out of the total isolates of pathogens including *K. pneumoniae* from sputum samples obtained from pneumonia patients (1 – 30 years) attending the University of Benin Teaching Hospital, Benin City.

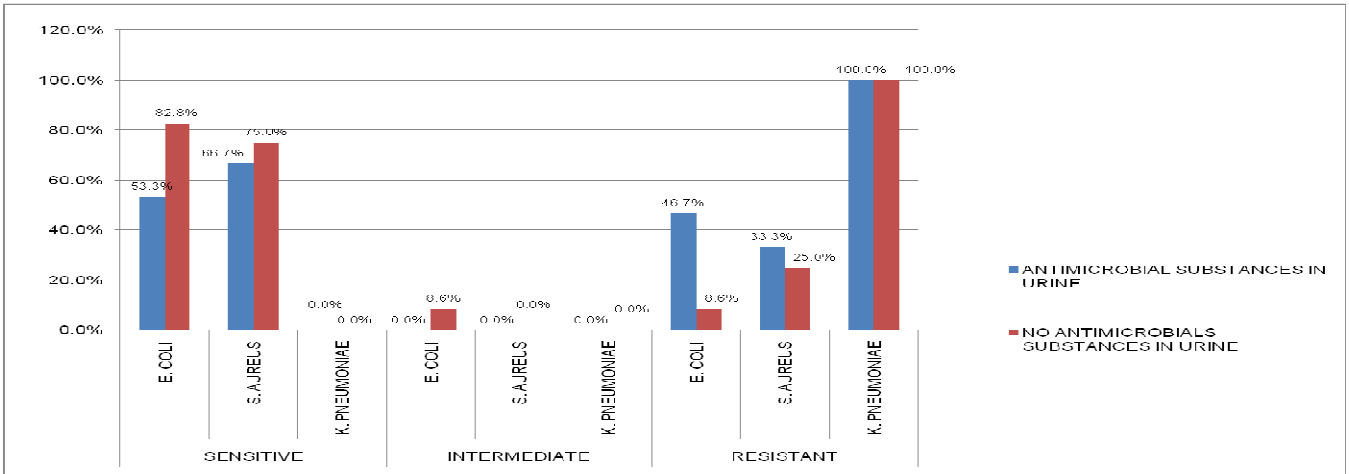

FIG. 5. EFFECT OF ANTIBIOTIC ABUSE THROUGH SELF MEDICATION ON THE RESPONSES OF 3 PATHOGENS TO CLINICALLY ADMINISTERED GENTAMYCIN THERAPY

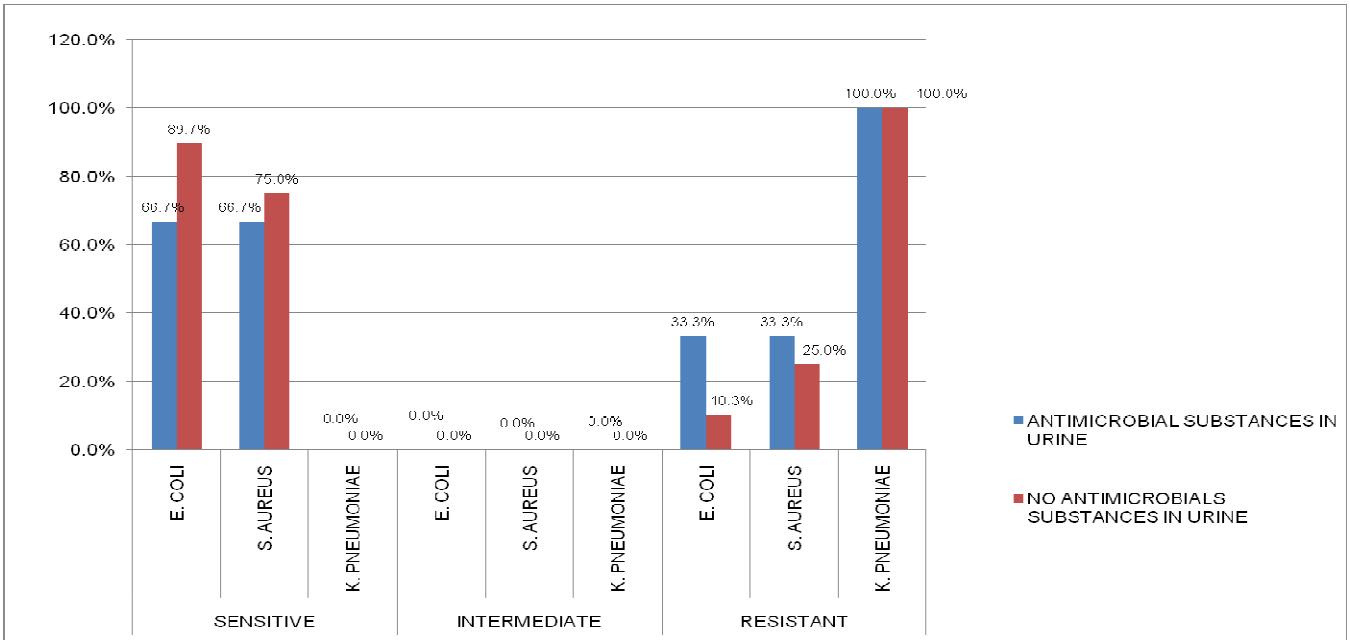

FIG. 6. EFFECT OF ANTIBIOTIC ABUSE THROUGH SELF MEDICATION ON THE RESPONSES OF 3 PATHOGENS TO CLINICALLY ADMINISTERED AUGMENTINN THERAPY

S. aureus showed total sensitivity to Imipenem (Fig 7). The incidence of resistance to the three antibiotics was higher from *E. coli* isolates from urine samples of self medicated patients than from *E. coli* isolates from urine samples from patients who did not self medicate.

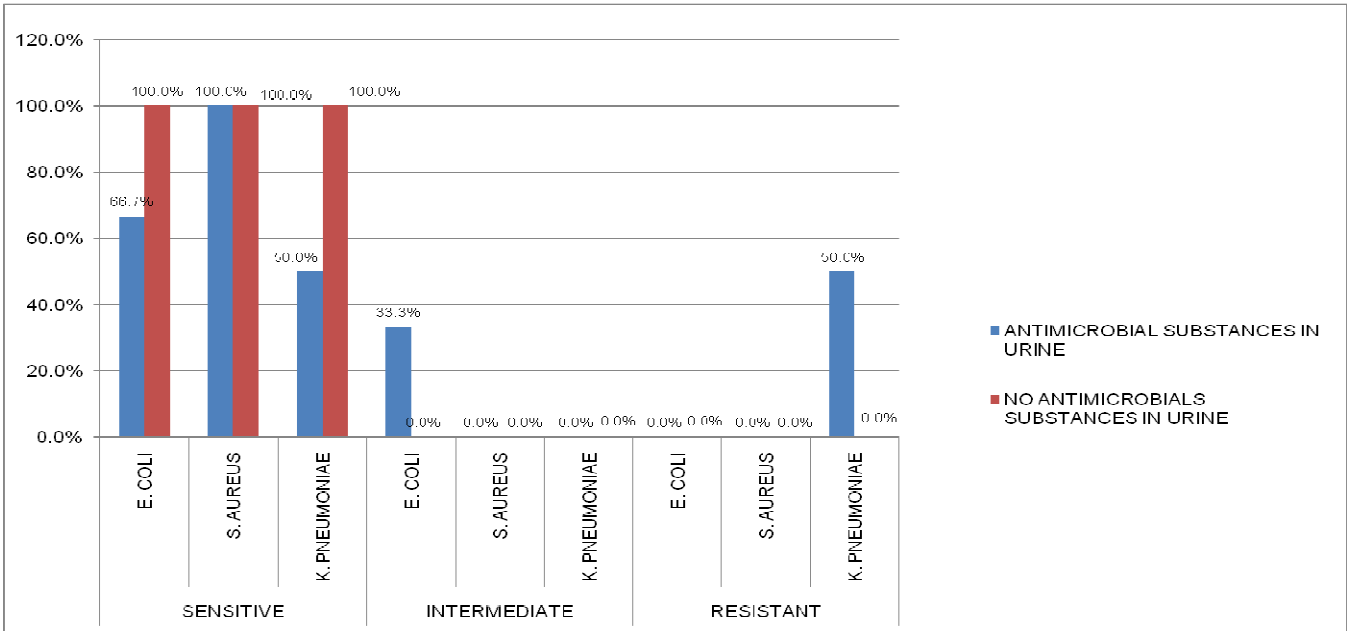

FIG. 7. EFFECT OF ANTIBIOTIC ABUSE THROUGH SELF MEDICATION ON THE RESPONSES OF 3 PATHOGENS TO CLINICALLY ADMINISTERED IMIPENEM THERAPY

E. coli was totally resistant to Amoxicillin (Fig 8). Oteo *et al* (2008) observed increased Amoxicillin–Clavulanic Acid (AMC) resistance of *E. coli* isolates from blood isolates from 42 Spanish hospitals and noted that this is of serious concern because AMC is the first-choice antimicrobial treatment for many invasive *E. coli* infections. Oteo *et al's* study (2008) showed that this development corresponded with increased consumption of AMC at the community level. In urinary infections, previous treatment with AMC is a risk factor for the development of AMC resistance.

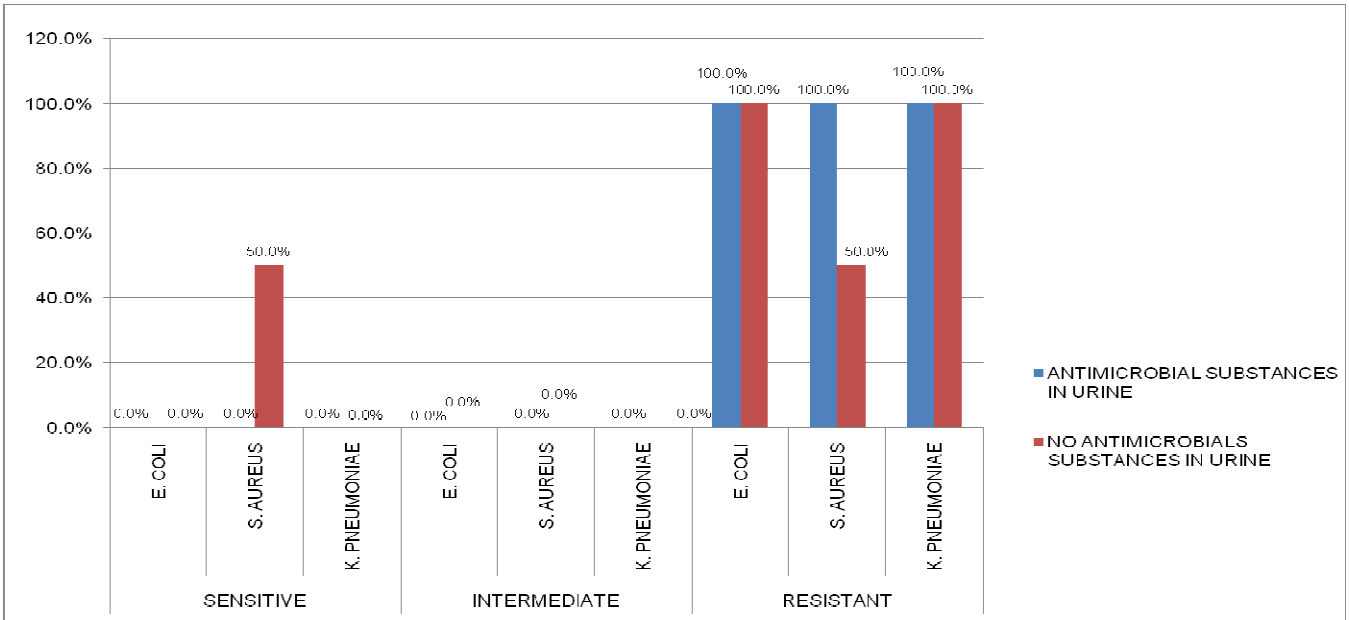

FIG 8. EFFECT OF ANTIBIOTIC ABUSE THROUGH SELF MEDICATION ON THE RESPONSES OF 3 PATHOGENS TO CLINICALLY ADMINISTERED AMOXICILIN THERAPY

S. aureus isolates from urine samples of patients who had not self medicated with antibiotics showed a higher susceptibility (75%) to Augmentin (Fig 6) than *S. aureus* isolates from urine samples of self medicated patients. *S. aureus* isolates from urine samples of self medicated patients showed higher incidence of resistance (66.7%) to Gentamycin, while *S. aureus* isolates from urine samples of non-self medicated patients showed 33.3% resistance to Gentamycin (Fig 5).

For Augmentin, the percentage of *S. aureus* isolates that showed susceptibility in urine samples of non-self medicated patients was 89.7% against 66.7% for isolates from samples of self medicated patients. The resistances of *S. aureus* isolates in samples from non-self medicated and self medicated patients were 10.3% and 33.3% respectively (Fig 6).

S. aureus isolates from both self medicated and non-self medicated patients showed total susceptibility (100%) to Imipenem. The response of *S. aureus* to Imipenem confirms Imipenem as a broad spectrum antibiotic which works effectively against both gram-negative and gram-positive organisms. The resistance of *S. aureus* to the other antibiotics could be attributed to the misuse of such antibiotics (Allen *et al*, 1997).

K. pneumoniae isolates from both self medicated and non-self medicated patients showed total (100%) resistance to Gentamycin (Fig 5) and Augmentin (fig 6).

The results confirm that antibiotic self medication prior to clinically prescribed antibiotic treatment has a significant influence on the response of bacteria to the clinically administered antibiotics.

Generally, more antibiotic self-medicating patients had resistant pathogens than non self-medicating patients.

Conclusions

Pathogens isolated from the urine of patients who self medicated showed higher percentage of resistance than those isolated from urine samples of patients who did not self medicate prior to the test. Even the broad spectrum antibiotics suffered some form of resistance from pathogens isolated from urine samples of self medicated patients.

The study shows that if care is not taken in the near future most of the pathogens would develop resistance against the antibiotics in use currently. Health officials must find effective means to control self-treatment with antibiotics in our communities. The public should be educated about the dangers involved in self-treatment with antibiotics specially when used as prophylactic drugs. A stricter control of the sale of antibiotics should be enforced so that the public access to them could be controlled.

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