CORRELATIONS BETWEEN ANTIBIOTIC INTAKE AND RESISTANCE OF SOME ENTERIC GRAM NEGATIVE BACILLI TO ANTIBIOTICS

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ABSTRACT

Some enteric Gram negative bacteria were isolated over a three – year (2006 – 2008) period from two human populations designated as population on therapy (OD) and population not on therapy (ND). Isolates were analyzed for susceptibility to a panel of ten antibacterial agents. Results showed that OD isolates were generally more resistant to test drugs than ND isolates but the differences were not significant at both 0.01 and 0.05 levels. Pearson correlation analysis showed that the correlation in resistances among the OD and ND isolates was systematic and significant at both 0.01 and 0.05 levels, suggesting that resistance emergence and sustenance may not be an exclusive consequence of intake and misuse of antibiotics.

KEY WORDS: Human population, Drug intake, Enteric bacteria, Drug resistance, Resistance correlation.

INTRODUCTION

Antibiotic resistance is perceived, worldwide, as a major crisis area of modern medicine (Varaldo, 2002). Increased morbidity and mortality are the most dramatic consequences but there are others (Livermore, 2003). One of these is that resistance, especially among the normal flora typified by the human enteric Gram negative bacilli, can lead to an increase in the incidence of diseases considering the anatomic position occupied by these organisms. This is most obvious for diseases in which antimicrobial treatment of sick persons or carriers is an important strategy in the prevention of additional cases of disease. Thus a person infected with a multidrug resistant strain of, for example, *Salmomella typhi* or *Escherichia coli* and who is not effectively treated will continue to pose a rick for transmission, in contrast to the patient infected with a susceptible strain for which treatment prevents transmission. A more subtle impact of drug resistance on the incidence of diseases occurs when a person receives an antimicrobial agent to which a potentially infecting or colonizing organism is already resistant. The antimicrobial drug, (in part) by killing competing organisms, provides a selective advantage that enables the resistant organisms(s) to cause disease(s), persist in the host for longer periods, or be spread more widely. In addition to these, the financial implications of antibiotic resistance are substantial. Treatment failures extend the length of hospital stay or demand repeated physician visits; hospital beds are blocked to new patients and productive time is lost. If new or hitherto reserved antibacterials are need as therapy, these are usually more expensive than previous regimens (Livermore, 2003).

Bacterial resistance to antibiotics has been attributed to many different factors with emphasis on the widespread use, misuse or overuse of antibiotics. Current thinking has referenced these as providing the selective pressure favouring propagation of resistant organisms (Livermore, 2003). This, however, conflicts with the information that resistant bacterial population existed long before the presence of any selective pressure occasioned by antibiotics (Hayes and Wolf, 1990). In view of this and the dearth of research reports linking direct intake of antibacterial agents with multiple drug resistance (MDR) among bacteria especially members of Enteric Gram negative bacilli, this work is designed to investigate the effect of active intake of antibacterial agents on the drug resistance pattern of enterics. This will be in comparison with those isolated from persons that have not (recently) taken antibiotics. The work is also aimed at investigating the correlation of MDR development between isolates from the two human groups.

MATERIALS AND METHODS

Isolation and Identification of Bacteria: This study was carried out over a three – year (2006, 2007 and 2008) period. Samples were collected from both apparently healthy persons at their homes and sick persons in hospitals. Donors were chosen based on information they gave as answers to structured questionnaires. Donors who had not taken antibacterial drugs three months before samples were collected were designated as population not on therapy (ND) while those on drugs within the time range were labeled population on therapy (OD) following the methods of Osterblad, *et al.,* (2000). Stool samples were collected in sterile plastic containers after informed consents were given by donors or their caregivers. Within 8 h of sample collection, a suspension of the formed or semiformed stool was made in 1 ml of presterilized peptone water (MERCK, 7228) (Cheesbrough, 2004). A loopful of each suspension was streaked on MacConkey agar (Lab M). Plates were incubated (24 h at 37^0C) and resultant

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colonies subsequently classified as lactose or non – lactose fermenters based on pigmentation (Levy *et al*., 1988). Colonies were selected based on the five – colony methods of Osterblad, *et al.,* (1995). Selected colonies were purified twice and subjected to standard morphological and biochemical tests and identified following the criteria of Krieg and Holt (1984) and Cowan and Steel (1965).

Antibiotic Resistance Determination: All bacterial isolates were tested for sensitivity to drugs by means of the disc diffusion method (CLSI 2006). The following Optun (Nig). antibiotic discs and concentrations were used: tarivid (10µg), peflacine (10µg), ciprofloxacin (10µg), augumentin (30µg), gentamicin (10µg), streptomycin (30µg), ampicillin (30µg), ceporex (10 µg), nalidixic acid (30 µg) and septrin (30 µg) . Standardized Mueller – Hinton broth cultures of isolates were assayed for sensitivity to these antibiotics on Mueller – Hinton agar plates as described earlier (Eze *et al*., 2009). Control plates were inoculated and incubated without discs. Following incubation (24 h at 37^0C) of test plates and measurement of inhibition zone diameters, susceptibility ranges were scored according to CLSI (NCCLS) (2006).

Statistical Analysis: Using the Statistical Package for Social Sciences (SPSS) Inc (444 N Michigan, USA), Analysis of variance (ANOVA), Pearson correlation and Post Hoc tests were carried out to determine any significant associations between antibiotic resistance (outcome variable) and sampling sites (OD and ND). The level of correlation of antibiotic resistance among the isolates from OD and ND was also determined. Significance level was scored at 0.05 and 0.01 levels (2 – tailed).

RESULTS

Detectable but not significant ($p > 0.05$) differences in resistances to test drugs were observed between isolates from OD and ND. Generally isolates from OD were more resistant to tarivid (3.14 %), peflacine (2. 98 %), ciproflox (2.83 %) augumentin (2.92 %), gentamicin (2.67 %), streptomycin (6. 44 %), ceporex (3. 77 %) and nalidixic acid (2. 98 %) than those from ND with corresponding percentage resistance of 1.07 %, 2. 09 %, 1.39 %, 2. 89 %, 1.50 %, 4.88 %, 2.58 % and 1. 82 % against the respective drugs (Fig. 1).

Individually, members of the various bacterial genera showed varied resistance patterns against the antibacterial agents. Species of *Citrobacter* from OD population were not resistant to the quinolones (peflacine and ciproflox) and ceporex while those from ND population showed percentage resistance of 3.03 % against both peflacine and ciproflox, and 4.55 % against ceporex (Fig 2). OD isolates of *Enterobacter* spp were more resistant to all the test drugs except streptomycin, ceporex and nalidixic acid than ND isolates (Fig. 3). Similarly, strains of *E. coli* isolated from OD were more resistant to gentamicin and streptomycin but less resistant to the rest of the drugs than ND isolates (Fig. 4).

With the exception of resistance to peflacine and augumentin, OD isolates of *Klebsiella* spp were more resistant to the test drugs than ND isolates (FIG.

5). *Proteus* spp isolated from ND showed higher percentage resistance to tarivid (3.45 %), peflacine (3. 45 %), and ceporex (6.89 %) than those from OD with resistance of 3.23 % against the three drugs. (Fig. 6). Resistance to augumentin, ciproflox, streptomycin, ampicillin and septrin was higher among *Pseudomonas* spp isolated from ND than those from OD but not against other test drugs (Fig. 7).

As shown in Figure 8 below, *Salmonella* OD isolates were more resistant to all the drugs but tarivid than the ND isolates of the same organism. Similalrly OD isolates of *Shigella* spp were more resistant to all the analyte drugs except ciproflox than ND isolates (Fig. 9). Pearson correlation analysis shows a high correlation of 0.950 between the level of resistance of *Proteus* spp (ND) and *Salmonella* spp (OD), indicating systematic or nonrandom variation between the two drug resistance patterns (Table 1). Table 1 also shows high correlations of resistance (0.811 – 0.963) among bacteria of the same genus isolated from OD and ND sources. For example, the correlation between OD and ND isolates of *Pseudomonas* spp is 0.938 and is significant at the 0.01 level. In a similar vein, there is high correlations of resistance among bacteria of different genera isolated from OD and ND populations. For instance, the resistance correlation between species of *Citrobacter* isolated from ND population and *Pseudomonas* spp from OD populations is 0.943.

DISCUSSION

Data obtained in this work comparing resistance of bacteria isolates from human population on therapy (OD) and those not on therapies (ND) indicate that OD isolates were generally more resistant to test drugs than ND isolates (Fig 1). This lends credence to the general belief that antibiotics and other bioactive substances select bacteria that eventually become more resistant to drugs than those that have not been exposed to selective agents (Levy, 1982, Corpet 1987, Levy *et al*., 1988). Other epidemiological studies have demonstrated the influence of antimicrobial use or misuse on the emergenc, persistence, and transmission of multidrug resistant bacteria (Cohen, 1992.). It is however informative that the differences in resistance among OD and ND bacteria isolates are not significant (both at 0.05 and 0.01 levels). Interesting also is that the correlation matrix shows high and significant correlation (0.688 – 0.963) of resistance among OD and ND isolates. This is an indication of systematic rather than random variation between the antibiotic resistance patterns of these two groups (Kelch and Lee, 1978). The import of this is that the rate and mechanisms by which these organisms resist these drugs are very similar if not the same and may be genetically mediated by the same or very closely related elements.

The results also suggest that resistance emergence and sustenance may not be an exclusive consequence of intake of antibacterial agents as has earlier been alluded (Salyers, 2004). Factors other than direct intake of and selection by antibacterial agents may be involved in the maintenance of drug resistance traits among bacteria. There is therefore the need for all stakeholders in medicine and public health to further broaden the search for the causes and mechanisms of development and transmissibility of resistant bacteria.
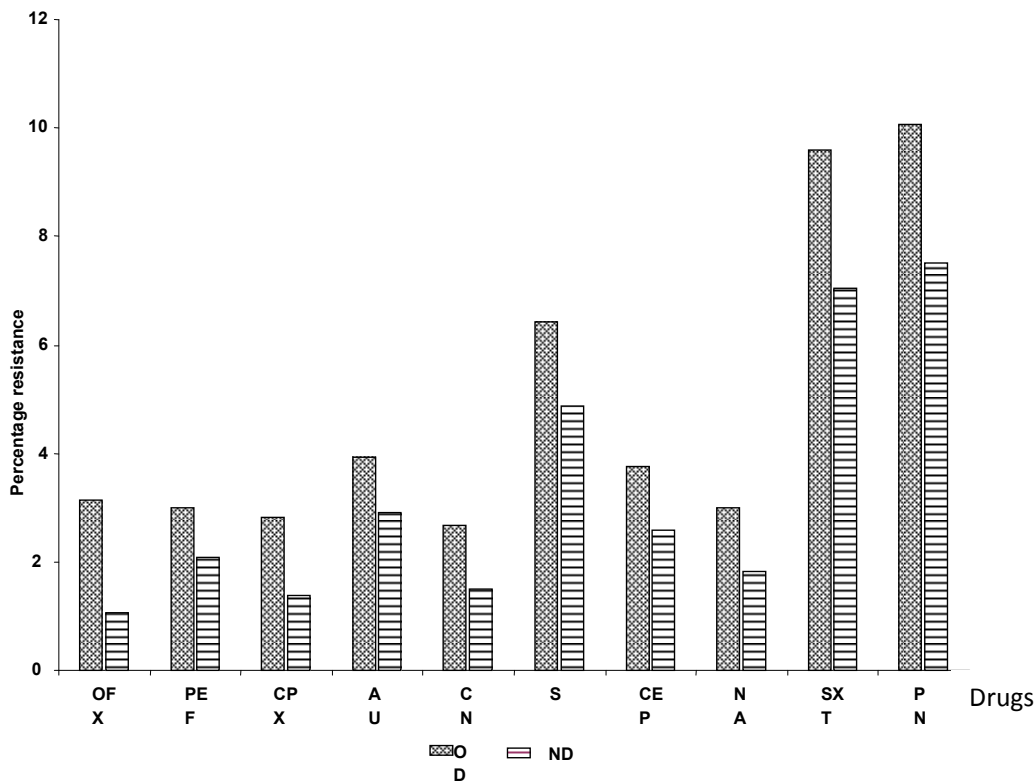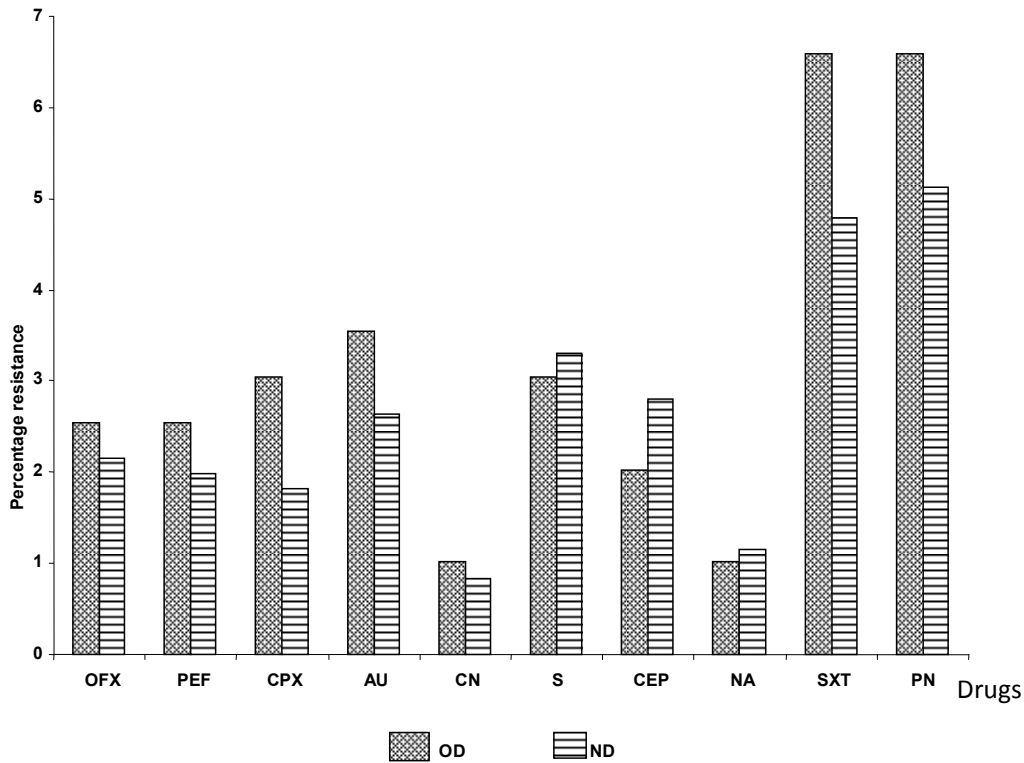
Fig.1: Generalized antibiotic resistance pattern of bacteria isolated from human population on therapy (OD) and those not on therapy (ND)

Key: OFX = Tarivid; PEF = Peflacine; CPX = Ciproflox; AU = Augumentin; CN = Gentamicin;
S = Streptomycin; CEP = Ceporex; NA = Nalidixic acid; SXT = Septrin, PN = Ampicillin
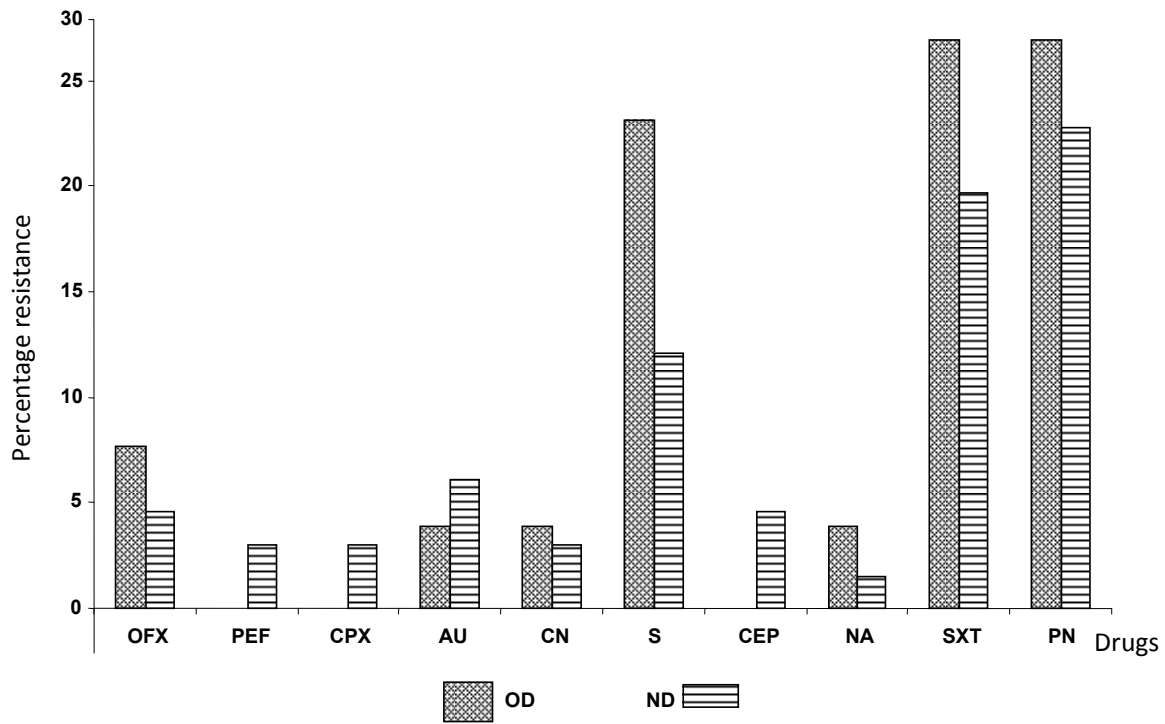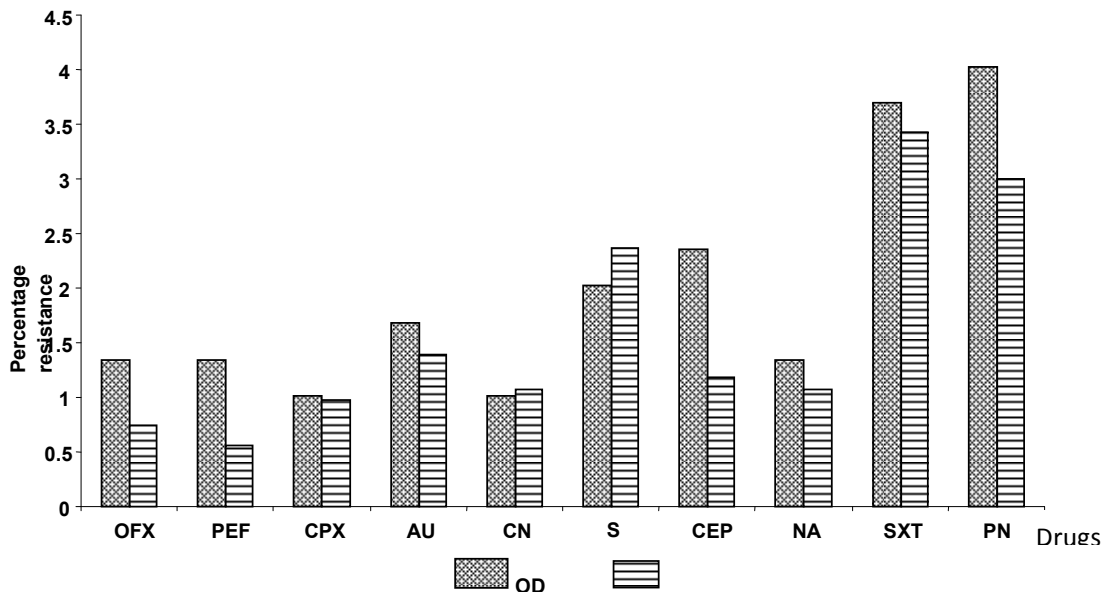
Fig.2: Drug resistance pattern of *Citrobacter* spp (n=92) isolated from human population on therapy (OD) and those not on therapy (ND)

Key: OFX = Tarivid; PEF = Peflacine; CPX = Ciproflox; AU = Augumentin; CN = Gentamicin; S = Streptomycin; CEP = Ceporex; NA = Nalidixic acid; SXT = Septrin, PN = Ampicillin

Key: OFX = Tarivid; PEF = Peflacine; CPX = Ciproflox; AU = Augumentin; CN = Gentamicin;
S = Streptomycin; CEP = Ceporex; NA = Nalidixic acid; SXT = Septrin, PN = Ampicillin.
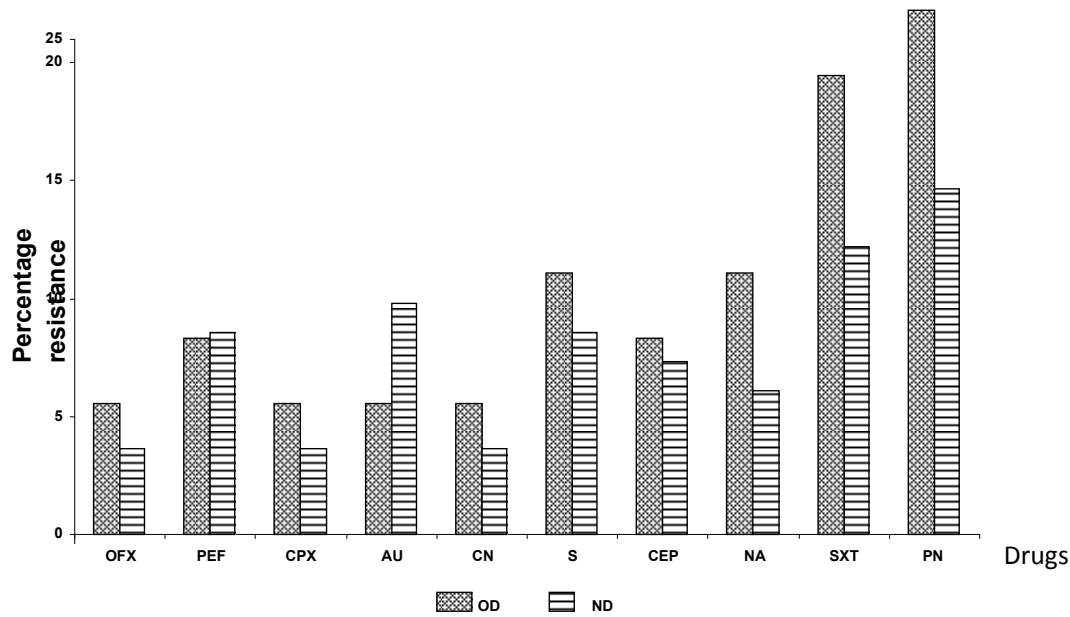
Fig. 3: Drug resistance pattern of *Enterobacter spp* (n=803) isolated from human population on therapy (OD) and those not on therapy (ND)

Fig. 4: Drug resistance pattern of strains of *E. coli* (n=1230) isolated from human population on therapy (OD) and those not on therapy (ND)
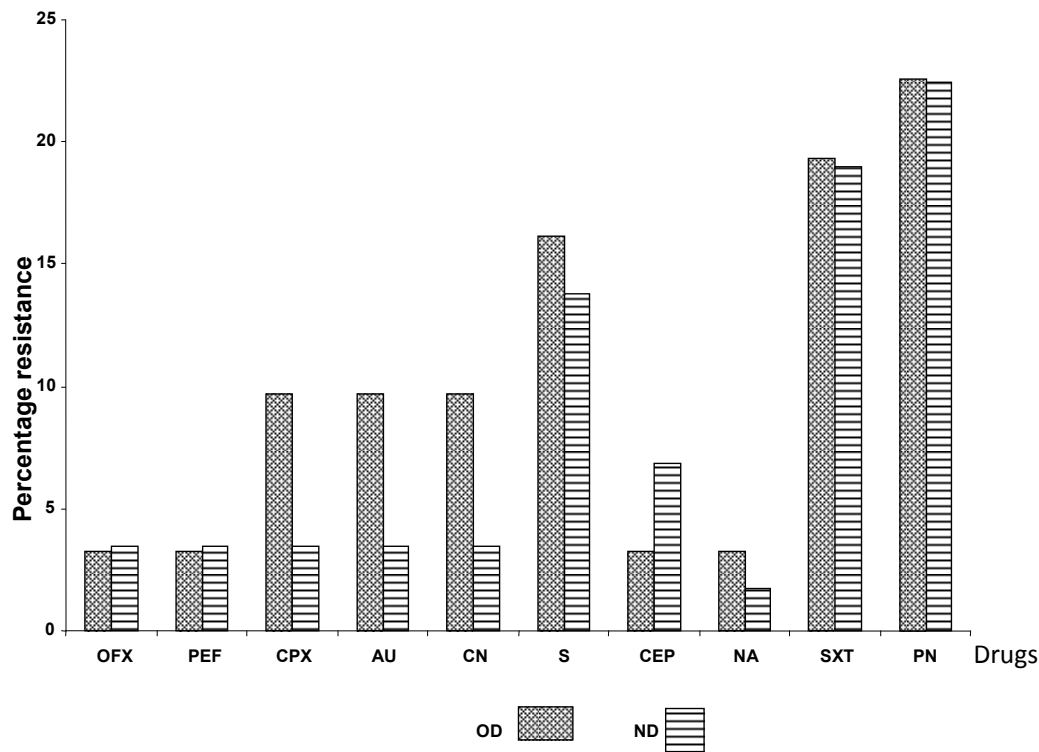
Key: OFX = Tarivid; PEF = Peflacine; CPX = Ciproflox; AU = Augumentin; CN = Gentamicin;
S = Streptomycin; CEP = Ceporex; NA = Nalidixic acid; SXT = Septrin, PN = Ampicillin.
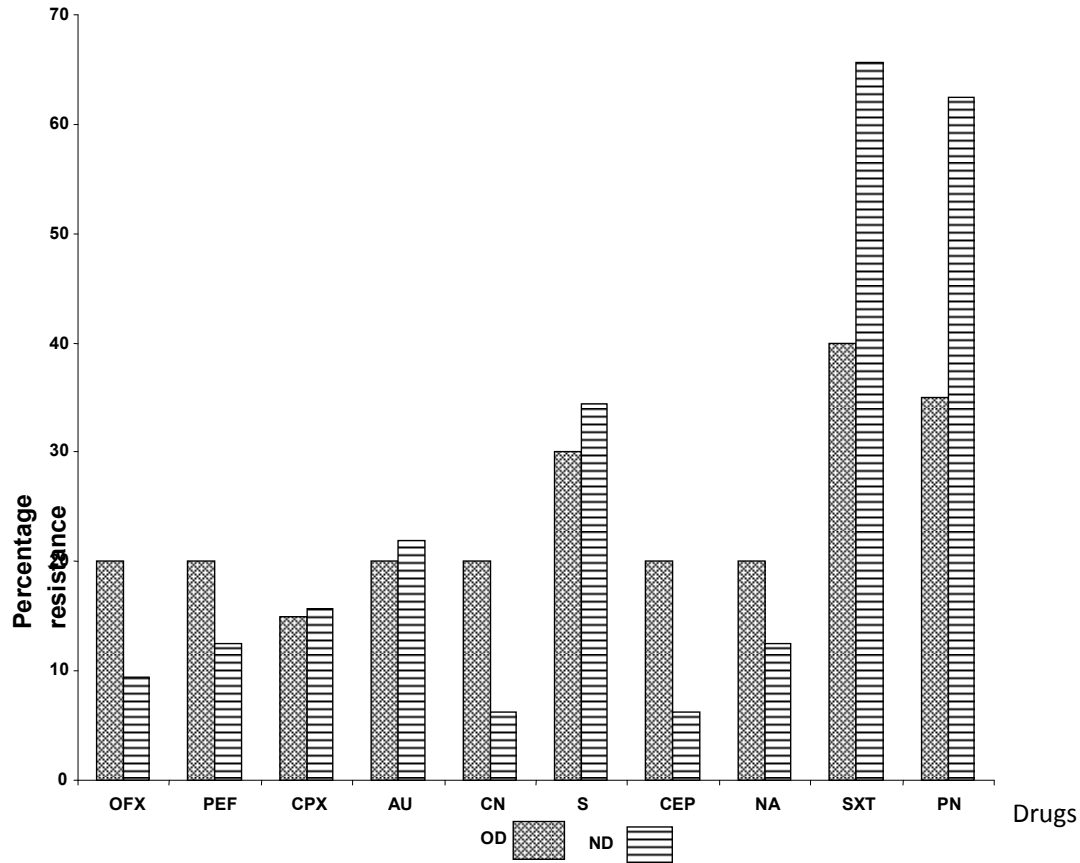
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Fig.5: Drug resistance pattern of *Klebsiella* spp (n=118) isolated from human population on therapy (OD) and those not on therapy (ND)

Key: OFX = Tarivid; PEF = Peflacine; CPX = Ciproflox; AU = Augumentin; CN = Gentamicin;
             S = Streptomycin; CEP = Ceporex; NA = Nalidixic acid; SXT = Septrin, PN = Ampicillin.
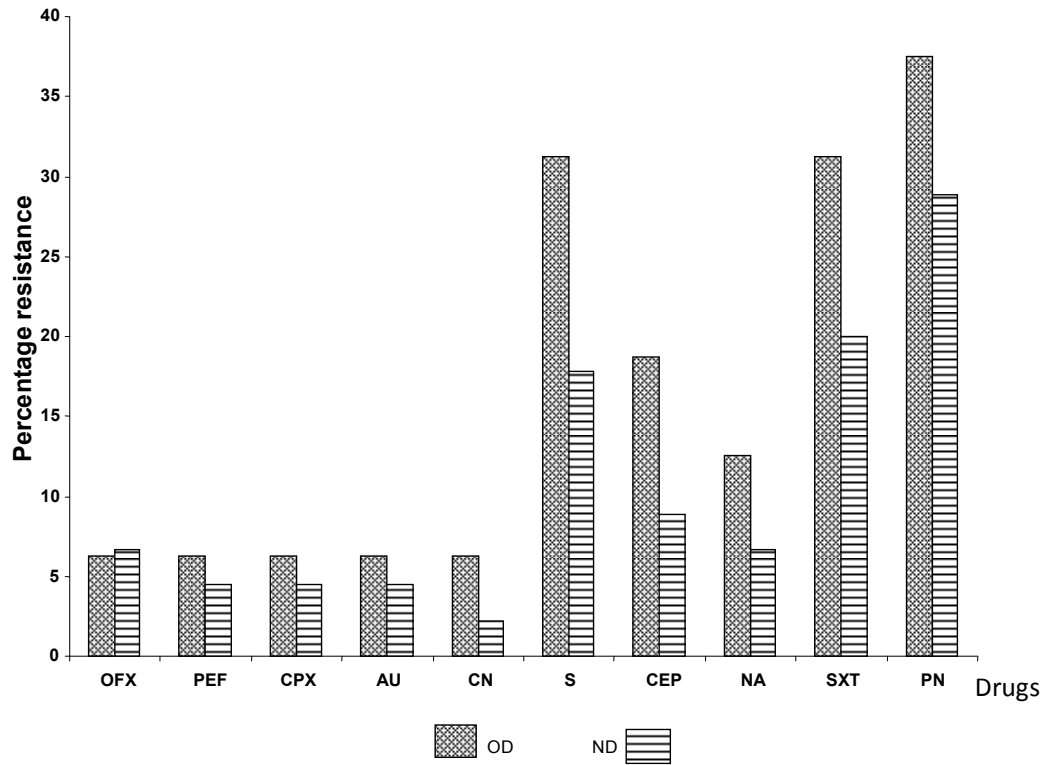

Fig.6: Drug resistance pattern of *Proteus* spp (n=89) isolated from human population on therapy (OD) and those not on therapy (ND)

Key: OFX = Tarivid; PEF = Peflacine; CPX = Ciproflox; AU = Augumentin; CN = Gentamicin;
             S = Streptomycin; CEP = Ceporex; NA = Nalidixic acid; SXT = Septrin, PN = Ampicillin.

Fig.7: Drug resistance pattern of _Pseudomonas_ spp (n=52) isolated from human population on therapy (OD) and those not on therapy (ND)

Key: OFX = Tarivid; PEF = Peflacine; CPX = Ciproflox; AU = Augumentin; CN = Gentamicin;
S = Streptomycin; CEP = Ceporex; NA = Nalidixic acid; SXT = Septrin, PN = Ampicillin.

Fig. 8: Drug resistance pattern of *Salmonella* spp (n=61) isolated from human population on therapy (OD) and those not on therapy (ND)

Key: OFX = Tarivid; PEF = Peflacine; CPX = Ciproflox; AU = Augumentin; CN = Gentamicin;
S = Streptomycin; CEP = Ceporex; NA = Nalidixic acid; SXT = Septrin, PN = Ampicillin.

Fig. 9: Drug resistance pattern of *Shigella* spp (n=57) isolated from human population on therapy (OD) and those not on therapy (ND)

Key: OFX = Tarivid; PEF = Peflacine; CPX = Ciproflox; AU = Augumentin; CN = Gentamicin;
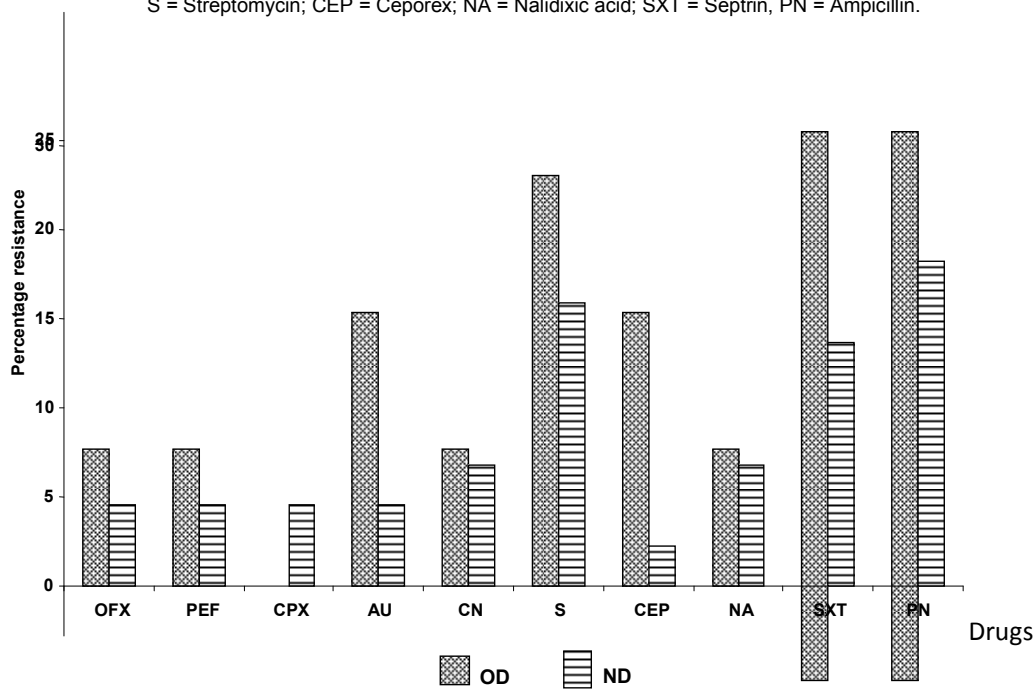S = Streptomycin; CEP = Ceporex; NA = Nalidixic acid; SXT = Septrin, PN = Ampicillin.

CORRELATIONS BETWEEN ANTIBIOTIC INTAKE AND RESISTANCE OF SOME ENTERIC

Table 1: Correlation Matrix of Antibiotic Resistance among some Enteric Gram-negative Bacteria Isolated from Humans on Therapy and those not on Therapy.

SOURCE	GROUP	HUMANS ON THERAPY (OD)						HUMANS NOT ON THERAPY (ND)									
		Citrobacter spp	*Enterobacter* spp	*Escherichia coli*	*Klebsiella* spp	*Proteus* spp	*Pseudomonas* spp	*Salmonella* spp	*Shigella* spp	*Citrobacter* spp	*Enterobacter* spp	*Escherichia coli*	*Klebsiella* spp	*Proteus* spp	*Pseudomonas* spp	*Salmonella* spp	*Shigella* spp
HUMANS ON THERAPY (OD)	*Citrobacter* spp	1															
	Enterobacter spp	.789**	1														
	Escherichia coli	.814**	.874**	1													
	Klebsiella spp	.844**	.805**	.923**	1												
	Proteus spp	.884*	.828**	.762*	.766**	1											
	Pseudomonas spp	.953**	.822**	.892**	.901**	.836**	1										
HUMANS NOT ON HERAPY (ND)	*Salmonella* spp	.898**	.713*	.888**	.889**	.801**	.894**	1									
	Shigella spp	.892**	.798**	.931**	.851**	.802**	.941**	.911**	1								
	Citrobacter spp	.939**	.922**	.939**	.905**	.914*	.943**	.899**	.931**	1							
	Enterobacter spp	.840**	.934**	.951**	.840**	.794**	.873**	.866**	.918**	.949**	1						
	Escherichia coli	.940**	.848**	.891**	.876**	.920*	.958**	.908**	.931**	.953**	.885**	1					
	Klebsiella spp	.712*	.828**	.886**	.842**	.708**	.802**	.768**	.884**	.849**	.877**	.791**	1				
	Proteus spp	.930**	.867**	.937**	.911**	.896**	.938**	.950**	.927**	.983**	.936**	.945**	.819**	1			
	Pseudomonas spp	.917**	.940**	.894**	.910**	.911**	.938**	.846**	.883**	.969**	.910**	.959**	.848**	.935**	1		
	Salmonella spp	.920**	.818**	.919**	.926**	.836**	.894**	.963**	.898**	.953**	.914**	.898**	.811**	.972**	.900**	1	
	Shigella spp	.949**	.688**	.728**	.832**	.898**	.871**	.885**	.811**	.880**	.735*	.878**	.687*	.892**	.863**	.903**	1

Key: * = Correlation is significant at the 0.01 level (2 – tailed); * = Correlation is significant at the 0.05 level (2 – tailed).

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