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# Multiple antibiotic resistance among Gram-negative bacteria isolated from hospital environment and in-patients

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ABSTRACT: A total of three hundred and eighty-nine gram-negative bacteria associated with nosocomical infections were isolated from the hospital environment, patients and hospital personnel for a period of 18 months in Ado-Ekiti State Specialist Hospital. Their susceptibility to commonly employed antibiotics and plasmid profiles were investigated. Clinical specimens were collected from patients with different cases of infections, swabs from inanimate objects were taken in various wards, and nasal samples of the hospital personnel were analysed. The most prevalent of the 197 bacterial isolates recorded from the clinical specimens included *Pseudomonas aeruginosa* 69 (35.0%); *Escherichia coli* 47 (23.9%); *Klebsiella* sp. 27 (13.7%); *Kblebsiella pneumoniae* 20 (10%) and *Salmonella typhi* 1(0.5%) being the least. While *Pseudomonas aeruginosa* 94 (48.9%)*; Proteus vulgaris* 43 (22.3%); *E. coli* 33 (17.2%) and *Klebsiella* sp. 10 (5.2%) were predominant among the 192 isolates recorded from the hospital environment, *Salmonella sp* and *Citrobacter freuidii* 2 (1%) occurred least. 96.4% of clinical and 90.1% of the hospital environmental isolates were multiple-antibiotic resistant types (MAR). They exhibited multiple resistance to the most commonly used antibiotics such asampicillin, tetracycline, streptomycin, nalidixic acid, colistin and cotrimoxazole. Plasmid profile analysis of typical resistant isolates showed DNA fragments which ranged from 4.1 to 53.5 kb among clinical isolates and less than 12.2 kb among the environmental isolates.

Keywords: Multiple antibiotic resistance; Antibiotic resistant gram-negative bacteria; Antibiotic resistant bacteria in hospital environment.

## Introduction

The emergence of bacterial resistance to chemotherapeutic agents is one of the major limitations to their successful therapeutic use by man (Holmberg et al., 1987). Apart from the economic burden, it also imposes serious limitation on the treatment of many bacterial infections, particularly hospital infections which can either stem from hospital personnel, hospital environment or from other patients (cross infection). Several bacterial species reportedly implicated in these infections are mostly resistant to commonly used antibiotics (Neu, 1992).

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Multiple resistant Gram-negative bacteria have posed a particular problem over the last decade (Gould, 1994). The development of resistant strain has reportedly been traced to certain environmental and clinical factors which include: Misuse of antibiotics through self-medication; sales of faked drugs and ease of availability of these drugs. Some of the clinical factors include severity of patients illness, length of stay in hospital, degree of immuno-suppression, instrumentation, over-crowding in poorly designed patient care unit and the quality of the antibiotics employed (Michael, 1989).

Prophylatic use of antibiotics, especially broad-spectrum antibiotics, aids rapid spread of resistance. resistance in Gram-negative bacteria may be plasmid or chromosomally mediated resulting from exposure to inducer compounds or by selection of stably repressed mutant (Casewell et al., 1981; Datta et al., 1984; Duddley, 1996). The use of sub-therapeutic levels of antibiotics for prophylaxis and as growth promoters remains concern as the laws of evolution dictate the emergence of resistant bacteria to practically any antibiotics. This study reports the antibiotic resistance and plasmid profiles among gram-negative isolates from hospital environment and in-patients.

## Materials and Methods

*Sample Collection*

Clinical specimens were collected from 375 hospital patients diagnosed with various cases of infections made up of 200 urine, 70 faecal, 30 post surgical wounds, 20 sputum samples, 30 urethral swabs (HVS) and 15 swabs. Samples collected from the hospital environment included swabs of scrubbled inanimate objects in various wards, such as sinks, louvers, bed sheet and stead, mattress, air conditioner vent, stretcher, window blind, personnel tables and chairs, nasal swabs and fingerprints of some medical personnel were obtained appropriately. Drinking water supplies (borehole and tap) within the hospital were examined and analysis of an immediate environment around the surgical ward was carried out.

*Bacteriological Analysis*

Samples were streaked on MacConkey agar, blood agar and nutrient agar as appropriate. Stool samples were enriched in buffered peptone water incubated at 37°C for 18 to 24 hours and subsequently inoculated onto *Salmonella-Shigella* agar.

Air samples were analysed by exposing prepared plates of nutrient agar in the surgical room for 5 seconds, after which the plates were covered and incubated. All plate cultures were incubated aerobically at 37°C for about 48 hours. Bacterial isolates were identified according to standard procedures (Cowan, 1993).

*Antibiotic Susceptibility Test*

The standard disc diffusion method and zone-size interpretation chart of Kirby-Baeur (Baeur et al., 1996) using McFarland's standard was employed. The following concentrations of the antibiotics were used: ampicillin 25µg, tetracycline 30µg, chloramphenicol 10µg, ofloxacin 10µg, ciprofloxacin 5µg, cotrimoxazole 25µg, cefriazone 25µg, streptomycin 25µg, nitrofirantion 200µg, colistin 10µg and nalidixin acid 30µg. *Escherichia coli* (NCTC 10418) was used as a control.

*Analysis of Plasmid*

Plasmid analysis was carried out on selected multiply antibiotic resistant isolates using the procedure of Birnborn and Doly (1979). Plasmids were detected by eletrophoresis in 0.8% agarose slab gels in Tris-borate buffer. Gels were later stained with 0.5% ethidium bromide. bands were visualized on ultra violent trans-illuminator and photographs taken with a Polaroid camera. The molecular size of the plasmid DNA was estimated by reference to known molecular weight plasmids of *E. coli* strain V512 included as control.

## Results

The distribution and the frequency of three hundred and eight nine isolates comprising 192 clinical isolates and 197 isolates recovered from the inanimate objects and hospital personnel is depicted in Table 1. Eleven bacterial species made up of *Ps. aeruginosa, E. coli, Kl. pnemoniae, Salmonella sp., Proteus mirabilis, Proteus vulgaris, Salmonella typhi* and *Salmonella paratyphi A., Serratia marcescens* and *Enterobacter sp.* were detected. The environmental isolates consisted of 7 different species which included *Ps. aeruginosa, P. vulgaris, E. coli, P. mirabilis, Klebsiella sp., Salmonella sp.* and *Citrobacter freundii*.

*Pseudomonas aeruginosa* predominated in both sample sources and exhibited the highest frequency of occurrence with 69 (35%) in clinical isolates as against 55 (56.6%) in environmental isolates.

Table 2 represents the incidence of resistance to antibiotics. All isolates examined carried resistant determinants to at least one antibiotic. The incidence of resistance to ampicillin, tetracycline streptomycin and colistin by strains from the both sources was high in that order but more conspicuous in environmental isolates. While resistance was generally high to ampicillin, it was least against cefotixime with 6.4% in a clinical isolate of *E. coli* and 2.1% in an environmental isolate of *Ps. aeruginosa* (Table 2). Resistance to oflotarivid, cefriaxone was equally low in that order. However, the difference in their frequency of resistant strains from both sources was obviously not significant.

Sixty percent (60%) of *Enterobacter sp.,* all *Klebsiella pneumoniae, Proteus micrabilis, Serratia marcescens, S. typhi* and *S. paratyphi* A isolates showed resistance to ampicillin. The resistance to this antibiotic follows the same order in the same species of the environmental isolates (Table 2).

Table 3 details the prevalence of multiple antibiotic resistance strains among the clinical and environmental isolates studied, while their profiles of multiple antibiotics resistance pattern is presented in Table 4.

Among the 197 clinical isolates, 44 (21.8%) showed resistance to either colistin, tetracycline, streptomyciun or ampicillin. 52 (78.2%) demonstrated multiple antibiotic resistance in varying degree of which 52 (26.4%) showed resistance to three different antibiotics, 44 (22.3%) to four, 31 (15.7% to five, 11 (5.5%) to six, 12 (6.0%) to seven and 4 (2.0%) to eight different antibiotics (Table 4).

Seventy eight (40.6%) of 192 environmental isolates resistance to one of the following: ampicillin, colistin, nitrofurantoin or nalidixic acid. Of these, 114 (59.4%) belong to the multiple-R-type with 46 (23.9%) showing resistance to three different antibiotics, 32 (16.7%) to four, 27 (14.0%) to five, 6 (3.1%) to six and 3 (1.6%) to eight different antiobiotics (table 4).

Also in Table 4, 84% of the clinical strains of *Ps. aeruginosa* showed multiple antibiotic resistance which ranged between three to eight different antibiotics while 71.3% of the same strains from the hospital environment showed multiple resistance to between three and six different antibiotics. Meanwhile, 40.6% and 39.3% of the clinical and environmental strain of *Ps. aeruginosa* respectively developed resistance to only ampicillin thus single-R-type.

All the clinical isolates of *Serratia marcescens, Salmonella sp., S. typhi, S. paratyphi* A and *Enterobacter sp.* belong to the multiple-R-type. Likewise, all the environmental strains of *Salmonella* sp. showed resistance to all the antibiotics studied.

In all, 32 different antibiotic resistance patterns were observed ranging from 2 to 7 MAR combinations with 8 different patterns among the clinical isolates as against 24 different patterns in environmental isolates.

Table 5 depicts some of the clinical and environmental isolates harbouring plasmid with their sources and estimated molecular weight. Of the 65 randomly selected clinical bacteria that showed multiple resistance to different antibiotics, 14 harboured plasmids of varying sizes. Four of the bacteria isolated from urine of patients with urinary tract infection harboured 74kb sized plasmid. These included *S. typhi (1), Kl. pneumonae (2)* and *Ps. aeruginosa* (1).

Three strains of E. coli recovered from stool, HVS, and urine samples of patients diagnosed with generalized infection and one strain of *Ps. aeruginosa* detected from the urine sample of a patient with urinary tract infection harboured one plasmid each with 55.5Kb size.

A strain of *Kl. pneumoniae* recovered from the sputum of a patient with upper respiratory tract infection harboured three different plasmids of the sizes 2.7, 4.1 and 12.2Kb respectively (Table 5).

Among the 15 randomly selected environmental isolates that demonstrated multiple resistance to different antibiotics, plasmid <12.2kb was extracted in 2 strains of *Ps. aeruginosa* recovered from the settled plate in the surgical room and the personnel chair respectively and a plasmid of the size 55.5kb was harboured by a strain of *P. vulgaris* cultured from a sink in the paediatrics ward.

Table 1. Distribution and frequency of bacterial isolates from clinical specimens

| Medical Personnel and Inanimate objects | | | | Organisms Isolated and their Frequency | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | Number of samples examined | Number isolated | *Cit. freundii* | *Ps. aeruginosa* | *Klebsiella pneumonia* | *Klebsiella* sp. | *E. coli* | *Proteus vulgaris* | *Proteus mirabilis* | *Entero-bacter* spp. | *Serratia marces-cens* | *Salmo-nella* spp. | *S. typhi* | *S. para-typhi* | |
| Urine | 200 | 92 | - | 43 (11.0) | 11 (2.8) | - | 21 (5.4) | 1 (0.3) | 4 (1.0) | 5 (1.3) | 4 (1.0) | - | 1 (0.3) | 2 (0.5) | |
| Stool | 70 | 33 | - | 1 (0.3) | - | - | 15 (3.8) | 5 (1.3) | - | - | - | 12 (3.1) | - | - | |
| High Vaginal Swab | 30 | 18 | - | 8 (2.1) | - | - | 10 (2.6) | - | - | - | - | - | - | - | |
| Sputum | 20 | 12 | - | 2 (0.5) | 9 (2.3) | - | 1(0.3) | - | - | - | - | - | - | - | |
| Septic wound | 40 | 37 | - | 10 (2.6) | - | 27 (6.9) | - | - | - | - | - | - | - | - | |
| Ear Swab | 15 | 5 | - | 5 (1.3) | - | - | - | - | - | - | - | - | - | - | |
| Nurses' hand | 20 | 9 | - | 8 (2.1) | - | - | 1 (0.3) | - | - | - | - | - | - | - | |
| Doctors' hand | 10 | 9 | - | 7 (1.8) | - | - | - | 2 (0.5) | - | - | - | - | - | - | |
| Nurses' nasal swab | 5 | 5 | - | - | - | - | - | 5 (1.3) | - | - | - | - | - | - | |
| Doctors' nasal swab | 5 | 5 | - | - | - | - | - | 5 (1.3) | | | | - | - | - | |
| Bore hole water | 10 | 10 | - | 5 (1.3) | - | - | - | 4 (1.0) | - | - | - | 1 (0.3) | - | - | |
| Tap water | 10 | 6 | - | 4 (1.0) | - | - | 2 (0.5) | - | - | - | - | - | - | - | |
| Water drainage System | 5 | 5 | - | 3 (0.8) | - | - | 2 (0.5) | | | | | - | - | - | |
| Water cistern | 2 | 2 | | 1 (0.3) | - | - | - | 1 (0.3) | | | | - | - | - | |
| Scrub sinks | 21 | 21 | 2 (0.5) | 9 (2.3) | - | - | 4 (1.0) | 5 (1.3) | - | - | - | 1 (0.3) | - | - | |
| Louvres | 30 | 20 | - | 7 (1.8) | - | - | 6 (1.5) | 7 (1.8) | - | - | - | - | - | - | |
| Bed sheet | 50 | 20 | - | 8 (2.1) | - | - | 5 (1.3) | 6 (1.5) | 1 (0.3) | - | - | - | - | - | |
| Bed stead | 11 | 11 | - | 10 (2.6) | - | - | - | 1 (0.3) | - | - | - | - | - | - | |
| Matress | 10 | 10 | - | 3 (0.8) | - | - | 2 (0.5) | 5 (1.3) | - | - | - | - | - | - | |
| Air conditioner Vent | 6 | 9 | - | 5 (1.3) | - | 3 (0.8) | 1 (0.3) | - | - | - | - | - | - | - | |
| Stretcher | 7 | 7 | - | - | | 4 (1.0) | - | 1 (0.3) | 2 (0.5) | - | - | - | - | - | |
| Window blind | 5 | 5 | - | 5 (1.3) | - | - | - | - | - | - | - | - | - | - | |
| Personnel's table | 11 | 11 | - | 5 (1.3) | - | 1 (0.3) | 5 (1.3) | - | - | - | - | - | - | - | |
| Personnel's chair | 5 | 5 | - | 2 (0.5) | - | - | 2 (0.5) | 1 (0.3) | - | - | - | - | - | - | |
| Male medical Ward | 3 | 3 | - | 2 (0.5) | - | - | - | - | - | - | 1 (0.3) | - | - | - | |
| Female surgical Ward | 3 | 3 | - | 2 (0.5) | - | - | - | - | 1 (0.3) | - | - | - | - | - | |
| Male surgical Ward | 3 | 3 | - | - | - | 1 (0.3) | 1 (0.3) | - | - | - | 1 (0.3) | - | - | - | |
| Operating room | 3 | 3 | - | 2 (0.5) | - | 1 (0.3) | - | - | - | - | - | - | - | - | |
| Paedetrics ward | 3 | 3 | - | 2 (0.5) | - | - | - | - | - | - | 1 (0.3) | - | - | - | |
| Antenatal /OPD | 3 | 3 | - | 2 (0.5) | - | - | - | - | - | - | 1 (0.3) | - | - | - | |
| Corridors | 4 | 4 | - | 2 (0.5) | - | - | 2 (0.5) | - | - | - | - | - | - | - | |

Table 2: Antibiotic resistance pattern of bacterial isolates from clinical sources and hospital environment*

| Antibiotics | *Ps. aeruginosa* | | *Klebsiella* spp. | | *E. coli* | | *P. vulgaris* | | *P. mirabilis* | | *Enterobacter* sp | | *Serratia marcescens* | | *S. typhi* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EI (n=94) | CI (n=69) | EI (n=10) | CI (n=27) | EI (n=33) | CI (n=47) | EI (n=43) | CI (n=6) | EI (n=4) | CI (n=4) | EI | CI (n=5) | EI (n=4) | CI (n=4) | EI (n=0) | CI (n=1) |
| Ampicillin | 70.2 | 92.7 | 100 | 74.1 | 93.8 | 9.5 | 83.7 | 83.3 | 75.0 | 100.0 | - | 80.0 | 100.0 | 100.0 | - | 100.0 |
| Tetracycline | 58.5 | 82.0 | 50.0 | 79.8 | 40.6 | 70.2 | 28.0 | 16.7 | 75.0 | 100.0 | - | 80.0 | 25.0 | 100.0 | - | 0 |
| Streptomycin | 36.2 | 41.3 | 60.0 | 18.5 | 53.1 | 27.7 | 25.6 | 66.7 | 50.0 | 50.0 | - | 0 | 25.0 | 100.0 | - | 0 |
| Colistin | 83.0 | 37.7 | 50.0 | 77.8 | 87.5 | 77.3 | 28.0 | 33.3 | 75.0 | 25.0 | - | 40.0 | 50.0 | 50.0 | - | 100.0 |
| Nalidixic acid | 34.0 | 53.6 | 80.0 | 37.0 | 34.4 | 31.9 | 51.2 | 83.3 | 25.0 | 0 | - | 40.0 | - | 100.0 | - | 100.0 |
| Nitrofurantrin | 11.7 | 30.4 | 15.0 | 11.1 | 59.3 | 25.5 | 25.6 | 83.3 | - | 0 | - | 40.0 | - | 0 | - | 0 |
| Cotrimoxazole | - | 34.8 | 40.0 | 11.1 | - | 34.0 | - | 16.7 | - | 0 | - | 20.0 | - | 100.0 | - | 100.0 |
| Ciproxin | 11.0 | 13.0 | 10.0 | 11.1 | 3.1 | 6.4 | 7.0 | 16.7 | - | 0 | - | 0 | - | 0 | - | 0 |
| Gentamicin | - | 11.6 | - | 7.4 | 3.1 | 34.0 | 7.0 | 16.7 | 25.0 | 0 | - | 60.0 | - | 50.0 | - | 100.0 |
| Oflotarivid | 2.1 | 1.4 | - | 0 | 3.1 | 8.5 | - | 50.0 | - | 0 | - | 0 | - | 0 | - | 0 |
| Ceftriaxone | - | 2.9 | - | 0 | 6.3 | 0 | 11.6 | 0 | - | 0 | - | 0 | - | 0 | - | 0 |
| Cefotaxime | 2.1 | 0 | - | 0 | - | 6.4 | - | 0 | - | 0 | - | 0 | 25.0 | 0 | - | 0 |

| *Salmonella* sp. | | *Citrobacter freundii* | | *Klebsiella pneumonia* | | *S. paratyphi* A | |
|---|---|---|---|---|---|---|---|
| EI (n=3) | CI (n=12) | EI (n=2) | CI (n=0) | EI (n=0) | CI (n=20) | EI (n=0) | CI (n=2) |
| 66.7 | 0 | 100.0 | - | - | 100.0 | - | 100.0 |
| 66.7 | 0 | 50.0 | - | - | 50.0 | - | 50.0 |
| 66.7 | 0 | 50.0 | - | - | 30.0 | - | 50.0 |
| 100.0 | 100.0 | 50.0 | - | - | 50.0 | - | 50.0 |
| 33.3 | 100.0 | - | - | - | 20.0 | - | 0 |
| - | 100.0 | - | - | - | 15.0 | - | 50.0 |
| - | 50.0 | - | - | - | 15.0 | - | 50.0 |
| - | 0 | - | - | - | 10.0 | - | 50.0 |
| - | 0 | 50.0 | - | - | - | - | 50.0 |
| 33.3 | 0 | - | - | - | - | - | 50.0 |
| - | 25.0 | - | - | - | 15.0 | - | 0 |
| - | 0 | - | - | - | 0 | - | 0 |

KEY
n = Number of strain tested
EI = Environmental isolates
CI = Clinical isolates
- = not determined
* = expressed in percentage

Table 3: Antibiotic resistant types among clinical and environmental bacterial isolates

| | Total Number of isolate | | Single-R-Type | | Antibiotics | | Multiple-R-Type | | Antibiotics | |
|---|---|---|---|---|---|---|---|---|---|---|
| Bacterial isolate | EI | CI | EI | CI | EI | CI | EI | CI | EI | CI |
| *Pseudomonas aeriginosa* | 94 | 69 | 35.1<br>2.1<br>2.1 | 15.9<br>-<br>- | col<br>amp<br>nit | amp | 71.3 | 84.1 | amp, tet, str, nit, nal, cip, gen | amp, tet,str, nal, col, cot, cit, gen, cro |
| *Escherichia coli* | 33 | 47 | 18.2 | 25.5 | amp | amp | 81.8 | 74.5 | amp, tet, str, nal, nit, col, cot | amp, str, nal, col, gen, nit, cro |
| *Klebsiella* sp | 10 | 27 | 60.0 | 44.4 | tet | col | 40.0 | 55.5 | amp, nal, tet, str, col, nit | amp, col, tet, str, nal, nit |
| *Proteus vulgaris* | 43 | 6 | 2.3<br>4.7<br>46.5 | - | col<br>nal<br>amp | - | 46.5 | 100.0 | nal, str, mp, tet, col, ctx, nit, cip | amp, str, nit, nal, gen, col, cot |
| *Proteus mirabilis* | 4 | 4 | 50.0 | 50.0 | col | amp | 50.0 | 50.0 | amp, col, gen, str, nal | amp, tet, str, col, nit, cip, nal |
| *Srratia marcescens* | 4 | 4 | 75.0 | - | amp | - | 25.0 | 100.0 | amp, col, tet, str | amp, tet, str, gen, col, nal, cot |
| *Salmonella* sp. | 2 | 12 | - | - | - | - | 100.0 | 100.0 | amp, col, tet, str, gen | cot, nal, nit, cro, col |
| *Salmonella typhi* | - | 1 | - | - | - | - | - | 100.0 | - | amp, tet, col, nal, cot, gen |
| *Salmonella paratyphi A* | - | 2 | - | - | - | - | - | 100.0 | - | amp, str, tet, cot, cip, gen, nit, ofx |
| *Enterobacter* sp | - | 5 | - | - | - | - | - | 100.0 | - | amp, tet, nal, cot, nit, gen, cro |
| *Klebsiella pneumonia* | - | 20 | - | 30.0 | - | amp | - | 70.0 | - | amp, tet, col, nal, nit, cip, cro |
| *Citrobacter freundii* | 2 | - | 50.0 | - | amp | - | 50 | - | Amp, tet, str, cip | - |

Key
EI = Number of environmental isolates tested,
CI = Number of clinical isolates strains tested
( ) = expressed in percentage
Amp – Ampicillin
Tet – Tetracycline
Nal – Nalidix acid
Nit - Nitrofunratoin
Gen – Gentamicin
Str - Streptomycin
Col - Cotrimoxazole
Ofx - Oflotarivid
Cip - Ciproxin
Cro - Ceftriaxone

Table 4: Profile of antibiotic resistance pattern of clinical and environmental bacterial isolates

| Isolates | Number of isolates | | Frequency of single and multiple antibiotic resistance types | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EI | CI | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | |
| | | | EI | CI | EI | CI | EI | CI | EI | CI | EI | CI | EI | CI | EI | CI |
| *Pseudomonas aeriginosa* | 94 | 69 | 37 | 11 | 19 | 17 | 21 | 23 | 13 | 8 | 4 | 3 | - | 6 | - | 1 |
| *Escherichia coli* | 33 | 47 | 6 | 12 | 14 | 12 | 7 | 8 | 5 | 10 | 1 | 2 | - | - | - | 3 |
| *Klebsiella* sp. | 10 | 27 | 6 | 12 | 1 | 9 | - | 4 | 3 | 2 | - | - | - | - | - | - |
| *Proteus mirabilis* | 4 | 4 | 2 | 2 | 1 | 1 | 1 | - | - | 1 | - | - | - | - | - | - |
| *Serratia marcescens* | 4 | 4 | 3 | - | - | - | - | 6 | 1 | - | - | 2 | - | 2 | - | - |
| *Salmonella* sp. | 2 | 12 | - | - | 1 | 4 | 5 | - | - | 2 | - | - | - | - | - | - |
| *Salmonella typhi* | - | 1 | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - |
| *Salmonella paratyphi A* | - | 2 | - | - | - | - | - | - | - | 2 | - | - | - | - | - | - |
| *Enterobacter* sp. | - | 5 | - | - | - | 1 | - | - | - | 1 | - | 2 | - | 1 | - | - |
| *Citrobacter freundii* | 2 | - | 1 | - | 1 | - | 0 | - | - | - | - | - | - | - | - | - |
| *Klebsiella pneumonia* | - | 20 | - | - | - | 7 | - | 1 | - | 3 | - | 1 | - | 2 | 2 | - |
| *Proteus vulgaris* | 43 | 6 | 23 | - | 9 | 1 | 2 | 2 | 5 | 2 | 1 | - | - | 1 | 3 | - |
| Total | 192 | 197 | 78 | 37 | 46 | 52 | 36 | 44 | 27 | 31 | 6 | 11 | - | 12 | 5 | 4 |

EI = Number of environmental isolates
CI = Number of clinical isolates

Table 5: Bacterial isolates harbouring plasmids and their molecular weights

| Clinical Isolates and the number examined | Source | Number of plasmids | Estimated size (kb) | Antibiotic resistance pattern |
|---|---|---|---|---|
| *Salmonella typhi* (1) | | 1 | 7.4 | Amp, tet, col, nal, cot, gen |
| *Klebsiella pneumonia* (5) | Urine | 1 | 7.4 | Amp, cip, col, nit |
| | | 1 | 7.4 | Amp, nal, cot |
| *Klebsiella pneumonia* (1) | Sputum | 3 | 2.7 | Amp, cip, col, cot |
| | | | 4.1 | Nal, nit, tet, str |
| *Escherichia coli* (13) | Stool | 1 | 12.2 | Nal, nit, cip, cot |
| | | | 55.5 | Amp, col, cot, cro, nal, nit, str |
| *Escherichia coli* (5) | HVS | 1 | 55.5 | Amp, col, tet |
| *Escherichia coli* (20) | Urine | 1 | 55.5 | Amp, str |
| *Pseudomonas aeriginosa* (20) | Urine | 1 | 7.4 | Amp, col, cot, tet |
| Environmental isolates | | | | |
| *Ps. aeriginosa* (5) | Surgical room | 1 | 12.2 | Amp, tet, str, nal, nit |
| *Ps. aeriginosa* (5) | Personnel' chair | 1 | 12.2 | Amp, tet, str, nal, nit |
| *Proteus vulgari* (5) | Sink | 1 | 55.5 | Amp, tet, nal, col, nit, cot |

## Discussion

Resistance to antibiotics by bacteria is a continuing and growing problem particularly among hospital bacterial pathogens, a number of hazards have emerged along with the benefits of antimicrobial therapy. The wide spread use of antibiotics for human therapy and in animal production had promoted the emergence and maintenance of multiple-antibiotic resistant bacteria. This also may have affected the changes in the ecology of bacterial infections and indeed also, the type of nosocomial infections and carries a strong prediction of therapeutic failure.

The isolates in this study varied in their resistance pattern to a number of the antibiotics investigated. While resistance to ampicillin and other commonly used antibiotics was generally hig, it was least against cefotaxine, this agent therefore being the most effective.

Forty three percent of strains of *E. coli* were resistant to ampicillin. This agreed with Ried et al (1988) who reported that 43-70% of the organisms isolated from Scottish patients in the early 1980 were resistant to ampicillin. Olayemi et al (1990) made a similar observation among the coliform bacteria isolated from hospital and urban waste water in Nigeria.

Antimicrobial resistance patterns revealed 32 different patterns. Strains of *Ps. aeruginosa* from both the clinical and environmental sources demonstrated the highest resistance pattern. This concurs with Odugbemi et al (1994) who described the organisms as being multiple-antibiotic resistant. Jiro (1992) reported a similar observation on *Ps. aeruginosa* as intrinsically resistant to many antibiotics due to its metabolic versatility and currently recognised as one of the leading causes of severe hospital acquired infections.

Resistance to some of the antibiotics examined in this study may indicate their therapeutic failure in the treatment of the infections from which the specimens were collected. The high level of resistance against these agents may, however, be attributed to their heavy use and abuse in the study area. This agreed with the earlier reports from Nigeria and other parts of Africa where self-medication and misuse of these agents are common (Obaseki-Ebor et al., 1987; Montefiero et al., 1989; Famurewa, 1992). The relative ease of accessibility to these antibiotics in some part of this country from diverse source such as pharmacists, patent medicine store and roadside stalls further worsen the control of drug abuse. Moreover, absence of enforced legislation against abuse of this sort of dugs aggravated the problem.

The susceptibility of some isolates to new, uncommon and or more expensive antibiotics such as cefotaxime, ceftriaxone and oflotarivid confirms the recent findings of Ogunsola et al (1999) who reported 100% sensitivity in *Ps. aeruginosa* strains to newer antibiotics which are highly expensive and beyond the reach of most Nigerians.

The high incidence of multiple antibiotic resistant organisms in this study may be the outcome of such abuses and selection pressure as reported earlier (Maniel et al., 1998; Bronzwaer et al., 2002; Dromigny et al., 2002). Such high incidence has been reported among members of Enterobacteriae (O'Brien et al., 1998). This may have implications on the treatment of infections caused by such aetiologic agents. Hence the monitoring of their effectiveness through prospective and continuous surveillance of antimicrobial resistance and sale has been recommended (Bronzwaer et al., 2002).

Consequent upon the discovery about 40 years ago that antibiotic resistance could be transferred among members of Enterobacteriae, attention has been focussed on infectious antibiotic resistant plasmids and the bacteria that carry them. The recovery of plasmids in some isolates showing multiple resistance type suggest that the multiple resistant genes in the isolates studied may be medicated by plasmids and thus has epidemiological significance. This agrees with Olukoya (1996) who reported multiple antibiotic resistance to tetracycline and isolated 12 different types of plasmids with molecular weight ranging between 3 to 180kb. This was further buttressed by Richard et al. (1981) who showed that plasmid exchange readily occurs both at intra- and inter-species levels in raw sewage systems.

The findings of this study present a potential health problem as the predominant organisms have increasingly been associated with outbreak of hospital acquired infections. This further strengthens the call for more rational and judicious use of antibiotics in Nigeria. Further studies using DNA probing and plasmid profiles; systematic laboratory control; continuous epidemiological surveillance of antibiotics resistance pattern; concerted effort of both physicians and the public and enforcement of legislation (national utilization of drug) against drug abuse may reduce the incidence of antibiotic resistance and the associated clinical problems.

## References


Bauer, A.W.; Kirby, W.N.M.; Sheries, J.C. and Turck, M. (1966). Antibiotic susceptibility testing ny a standardized disk method. Antimicrobial Agents and Chemotherapy. 5: 86 – 90.

Bimboin, H.C. and Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Aci Review 7: 1513.

Bronzwaer, S.L.A.M.; cars, O.; Buchholz, U.; Molstad, S.; Goettsch, W.; Veldhuijzen, I.K.; Kool, J.L.; Sprenger, M.J.W. and Degener, J.E. (2002). A European study on the relationship between antimicrobial use and antimicrobial resistance. Emerging Infectious Diseases. 8: 278 – 282.

Caswell, M.W. and Philips, I. (1981). Aspects of the plasmid-medicated antibiotic resistance and epidemiology of *Klebsiella* species. American Journal of Medicine 70: 459 – 462.

Cowan, S.T. (1993). Cowan and Steel's manual for the identification of medical bacteria. 4th Edition, Cambridge University Press, London, pp. 26 – 37.

data, N. and Nugent, M.E. (1984). Plasmids in wild strains of bacteria. In: A puhler and K.N. Timmis (eds.) Advanced Molecular genetics. Springerverlag, berlin.

Dromigny, J.A.; Rakoto-Alson, O.; Rajaonatahina, D.; Migliani, R.; Ranjalahy, J. and Mauclere, O. (2002). Emergence and rapid spread of tetracycline-resistant *Vibro cholera* strains, Madagascar. Emerging Infectious Diseases. 8: 336-338.

Dudley, M. (1996). Bacterial resistance mechanisms to beta-lactam antibiotics: resistance assessment of management strategies. Pharmacotherapy. 15: 95 – 145.

Famurewa, O. (1972). prevalence of urinary tract infection in women in Ado-Ekiti, Ondo State, Nigeria. L'Igiene Moderna. 97: 580 – 591.

Gould, T.M. (1994). Risk factors for acquisition of multiply drug-resistant gram-negative bacteria. European Journal of Clinical Microbiology and Infectious Diseases. 13 (Suppl.): 530 – 538.

Holmberg, S.D.; Solomon, S.L. and Blake, P.A. (1987). health and economic impacts of antimicrobial resistance. Review of Infectious Diseases. ??? 1065 – 1078.

Manie, T.; Khans, S.; Brozel, V.S.; veith, W.J. and Gouvos, P.A. (1998). Antimicrobial resistance of bacterial isolates from slaughtered and retail chickens in South Africa. letters in Applied Microbiology. 26: 253 – 258.

Montefiero, D.; Rotimi, V.O. and Adeyemi-Doro, F.A.B. (1989). The problem of bacteria resistance to antibiotics among strains from hospital patrients in Lagos and Ibadan, Nigeria. Journal of Antimicrobial Chemotherapy. 23: 641 – 651.

Nagayama, F.T.; Takigawa, K.; Yamagishi, Y., Kubo, A.; Yamaji, Y. and Jakara, T. (1992). Activity of antibiotics against resistant *Pseudomonas aeruginosa*. Journal of Antimicrobial Chemotherapy. 29: 539 – 546.

Neu, C. (1992). The crisis in antibiotic resistance. Science. 257: 1054 – 1073.

Obaseki-Ebor, E.E.; Akerele, J.O. and Ebea, P.O. (1987). A survey of antibiotic outpatient prescribing and antibiotic self-medication. Journal of Antimicrobial Chemotherapy. 20: 759 – 763.

O'Brien, T.F. and the members of the Task Force 2 (1987). resistance of bacteria to antimicrobial drugs. report of Task Force 2. Review of Infectious Diseases. 9 (Suppl.): 5244 – 5260.

Odugbemi, T.; Oduyebo, O. and Animashaun, T. (1994). Typhoid fever. Microbiological aspects. Nigerian Postgraduate Medical Journal. 1(2): 39 – 43.

Ogunsola, F.T.; Kosah, C.N.; Odugbemi, T. (1997). Antimicrobial resistance in Nigeria: an overview. Journal of Hospital medicine. 7(1): 57 – 61.

Olayemi, A.B. and Opaleye, B.F. (1990). Antibiotic resistance among coliform bacteria isolated from hospital and urban waste waters. World Journal of Microbiology and Biotechnology. 6: 285 – 288.

Olukoya, D.K.; Daini, O.A. and Niemogba, G. (1996). Preliminary epidemiological studies on tetracycline resistant plasmid isolated from enteric bacteria in Nigeria. Tropical Geography and Medicine. 45: 117 – 120.

Parry, M.T. (1989). Epidemiology and mechanism of antibacterial resistance. American Infectious Control. 340: 286 – 294.

Stein, C.M.; Todd, W.T.A.; Parirenyatwa, D.; Chakonda, J. and Dizuani, A.G.M. (1984). A survey of antibiotic use in Harare Primary health care Clinics. Journal of Antimicrobial Chemotherapy. 14: 149 – 156.