Antibiotic resistance among coliform bacteria isolated from hospital and urban wastewaters

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The mean count of coliforms/ml in urban and hospital drains were $68.9 \times 10^4$ and $48.4 \times 10^4$, respectively. *Klebsiella* predominated amongst the 16 coliform species that were differentiated; *Escherichia coli* occurred with highest frequency. All the isolates were resistant to at least one antibiotic and resistance to ampicillin, tetracycline, and chloramphenicol was particularly high. Multiple antibiotic resistant strains were common in both environments—85% and 85% for hospital and urban sources, respectively. The difference in the frequency of resistant strains from the two sources was not significant. 20% of strains from the two sources were multiply resistant to six antibiotics. The role of untreated wastewater in the maintenance and dissemination of resistant coliforms and its public health significance are highlighted.

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Introduction

The coliform bacteria are widely distributed and ubiquitous in the sense that they are not only found in the intestinal tract of man and higher animals. Some coliforms have long been established as free living and there is increasing evidence that they have always been part of the natural microflora of the tropics (Rivera et al. 1988).

Although ordinarily commensals as a part of the intestinal flora, coliforms are potentially pathogenic elsewhere in the body where they produce pathogenic and diarrhoeal diseases, especially in children, elderly people and those debilitated by other illnesses (Richards et al. 1981). *Citrobacter* and *Serratia* are common in hospitalized patients as 'superinfections'.

Along with the benefits of antimicrobial therapy, a number of hazards have emerged. The widespread use of antibiotics for human therapy and in the production of poultry, eggs and pork, has promoted the emergence and maintenance of multiple antibiotic resistant coliforms. This has also resulted in changes in the ecology of bacterial infections and in the types of nosocomial infections.

The discovery 30 years ago that drug resistance could be transferred between members of the Entercobacteriaceae has focused attention on infectious drug resistant plasmids and the bacteria that carry them. This phenomenon, in conjunction with the selection pressure imposed by antibiotic usage, has increased the incidence of pathogenic strains that have acquired antibiotic resistance (Routman et al. 1985). Richards et al. (1981) have demonstrated that this plasmid exchange readily occurs both at intra- and interspecies levels in raw sewage systems.

In this study, the incidence of antibiotic resistance among coliforms isolated from the urban and hospital sewers was investigated. The distribution of species among the enteric bacterial flora was determined, including their antibiotic resistance patterns.
Le comptage moyen de coliformes par ml dans des égouts urbains ou d'hôpital était respectivement de $68.9 \times 10^4$ et de $48.4 \times 10^4$. *Klebsiella* prédominait parmi les 16 espèces de coliformes différenciées; *Escherichia coli* apparaissait avec la fréquence la plus élevée. Tous les isolats étaient résistants à au moins un antibiotique et la résistance à l'ampicilline, la tetracycline et le chloramphénicol était particulièrement élevée. Des souches résistantes à plusieurs antibiotiques étaient communes dans les deux environnements—85% et 65% respectivement pour les origines urbaines et d'hôpital. La différence dans la fréquence de souches résistantes provenant des deux origines n’était pas significative. Vingt pour-cent des souches provenant des deux origines présentaient une résistance multiple aux six antibiotiques. On a mis en évidence le rôle de l’eau résiduaire non traitée dans le maintien et la dissémination de coliformes résistants et de sa signification pour la santé publique.

**Materials and Methods**

**Sampling Sites**

Sixteen different urban sewage drain sites in areas of both high and low population density were selected. These consisted of domestic, combined and anonymous sewage types, i.e. domestic, industrial and storm run-off effluents. The University of Ilorin Teaching Hospital and three other private clinics were chosen to represent the hospital environment. Nine locations within their premises, including sewer drains from the diagnostic laboratory, wards, paediatric and dental units, kitchen and laundry, were sampled.

**Sampling Collection and Bacteriological Analysis**

Wastewater samples were collected in sterile 500 ml sample bottles according to Standard Methods (American Public Health Association 1981). The collection was usually made in early hours of the morning and transported to the laboratory within 2 hours for analysis. A total of 52 samples were taken, comprising 21 from hospital and 31 from urban sites.

The samples were serially diluted, plated on Endo-agar using the pour-plate method and incubated for 24 h at 37°C. Colonies showing typical coliform morphology (pink-rose-red with or without metallic sheen) were enumerated, restreaked for purity on cysteine/lactose/electrolyte-deficient (CLED) agar. Lactose-fermenting colonies were differentiated by the indole, methyl red, Voges-Proskauer and citrate (IMVIC) test series. Species were further confirmed by the reactions in KIA, motility/indole/urea media. Identification was according to Cowan & Steele (1974). The pure isolates were subcultured and maintained on nutrient agar slopes as stocks.

**Antibiotic Susceptibility Test**

The standard disc diffusion method and zone-size interpretation chart of Kirby-Baer (1966) was used. The following concentrations of the antibiotics, (in μg) were used: ampicillin, 25; tetracycline, 30; chloramphenicol, 10; gentamicin, 10; ofloxacin, 10; ciprofloxacin, 5; co-trimoxazole, 25; and streptomycin, 25. A total of 130 randomly selected coliform species were tested. *Escherichia coli* (NCTC 10418) served as control and results obtained were classified as resistant or sensitive.

**Results**

On the basis of colonial morphology, a total of 258 coliform isolates comprising 145 from urban sewers source and 113 from hospital sewers were identified. The mean coliform counts/ml for the former was $68.9 \times 10^4$. Seventeen species were differentiated, 16 of which were recoverable from the hospital sewers as against 14 from the urban sources. The genus *Klebsiella* predominated in the two environments. Eight species including *Klebsiella pneumoniae*, *K. aerogenes*, *K. oxytoca*, *K. edwardsiella*, *K. ozaenae*, *K. rhinoscleromatis*, *K. atlanta* and a *Klebsiella* sp. were identified. Other genera identified included *Escherichia*, *Serratia*, *Enterobacter*, *Hafnia* and *Edwardsiella*. The last was recovered only from hospital wastewater while *K. atlanta* was not found in urban sewers. *E. coli* exhibited the highest frequency of occurrence. Table 1 details the incidence of resistance to antibiotics. All isolates tested carried resistant determinants to at least one antibiotic. The incidence of resistance to ampicillin, tetracycline and chloramphenicol by strains from the two sources was high. However, the difference in the frequency of resistant strains from the two environments was not significant ($\chi^2 = 0.34; P = 0.05$).

Resistance patterns showed that 35% of the urban coliforms were resistant to single antibiotics while the other 65% were multiply resistant. Among the hospital