INHIBITORY EFFECTS OF SELECTED DISINFECTANTS AND ANTISEPTICS ON SOME RESISTANT STRAINS OF *PSEUDOMONAS AERUGINOSA.*

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ABSTRACT

The effects of commercial preparations of some disinfectants and antiseptics on thirteen resistant strains of *Pseudomonas aeruginosa* were investigated. The chemical agents used: Dettol, Septol, Z germicide and 70% ethanol common in use in Nigeria for household and laboratory disinfection showed good antibacterial efficacies resulting in at least 92% killing when the cells were exposed to the agents within a period of 10minutes. Antibacterial activities of the chemical agents are in the order: 70% Alcohol>Dettol>Septol>Z germicide. The sensitivity of the isolates to commonly used antibiotics was evaluated using commercial antibiotic discs. About 23% of the isolates were resistant to gentamicin, while 54% showed resistance to streptomycin. In all, 23% were resistant to all the antibiotics tested. Although, all the isolates were resistant as obtained from the discs, different pattern of resistance were obtained based on the source of the isolates. The soil isolates were less resistant to the antibiotics than the clinical isolates as they were susceptible to streptomycin and gentamicin. The extent of resistance shown by the isolates has serious public health implications.

KEYWORDS: Disinfectants, antiseptics, resistance pattern, *Pseudomonas*, resistant isolates.

INTRODUCTION

Studies on the resistance of pathogenic organisms to antimicrobial agents in use in Nigeria (Kolawole, 1983, 1984, 1985; Kolawole et al., 1988; Oloke, 2000) have been mainly on the clinical isolates of *Staphylococcus aureus* and *Escherichia coli*. The study has not been extended to members of the genus *Pseudomonas*, which is an important pathogen of man. However, since bacterial sensitivity and resistance vary considerably from place to place with their own particular resistance problems (Greenwood, 1989), it is important that studies on sensitivity and resistance should be extended to organisms isolated from other areas apart from the hospitals and patients. An important area of consideration in this regard is soil, whether such is contaminated or not. The investigation may permit to establish the resistance pattern of different strains of the same organism to antimicrobial agents. The effects of the source of isolation of strains of *Pseudomonas aeruginosa* on their susceptibility to antibiotics, and commercial preparations of disinfectants and antiseptics common in use in Nigeria were evaluated.

MATERIALS AND METHODS

Microorganism

Eight clinical and five soil isolates of *Pseudomonas aeruginosa* were used in this study. The clinical isolates were obtained from Baptist Medical Centre, Ogbomoso, while the soil isolates were obtained from oil contaminated soils (Ogbomoso, Southwest, Nigeria, and Ilorin, Northcentral, Nigeria).

Antimicrobial agents

Commercial preparations of Dettol, Septol and Z germicide were obtained from a local chemist shop in Ogbomoso, Southwest Nigeria. Solution of 70% ethanol was prepared in the laboratory when required. The descriptive analysis of the antimicrobial agents is presented in Table 1. The concentrations used were those at the middle of the ranges recommended by the manufacturers. All solutions were prepared in sterile distilled water and kept at 4°C until required.

Measurement of the rate of killing

This was done by the method earlier described (Kolawole, 1984, 1985). Briefly, 0.5ml of a known concentration of each organism suspension was added to 4.5ml of the disinfectant held at room temperature and mixed thoroughly. Samples (0.5ml) were transferred at predetermined intervals into 4.5ml of recovery medium (3% thioglycollic acid in nutrient broth), shaken well, diluted serially and then plated on nutrient agar. The recovery medium neutralizes the effects of disinfectant carry-overs from the test suspensions. The plates were incubated at 37°C for 48hrs. The control experiment consisted of observations of 0.5ml of organism suspension in 4.5ml of sterile distilled water.

Antibiotic sensitivity pattern

This was investigated using H Abioscs (Abtek Biological, Liverpool, UK) containing the following: Ampicillin, (Amp) 10µg; Cloxacillin (Clox) 5µg; Gentamicin, (Gen) 10µg; Penicillin, (Pen) 1 unit; Streptomycin, (Str) 10µg; Chloramphenicol, 10µg and Tetracycline, (Tet) 10µg. After incubation, the plates were examined for zones of inhibition and interpreted accordingly (Chortyk et al., 1993).

RESULTS AND DISCUSSION

The identity of the test organisms used in the study, and the percentage of organisms killed in 10minutes by the disinfectants and antiseptics are as shown in Table 2. The sensitivity patterns of the organisms to the antibiotics are obtainable from Table 3. All the isolates were found to be resistant to Ampicillin, Chloramphenicol, Cloxacillin, Tetracycline and Penicillin.

The results showed that all the four chemical agents were effective against all the strains of *Pseudomonas aeruginosa* with 70% ethanol being the most active, followed by Dettol, Septol and then Z germicide. All of them showed at least 92% viability reductions within 10minutes. These observations suggest that these chemical agents would be effective for disinfections both in the house and laboratories.

The observed difference in the activity of the chemical agents could be attributed to the uses for which they are intended, which determine the type and the amount of active ingredients incorporated into the commercial products. Earlier, Kolawole (1984, 1985) reported the inhibition of the antibacterial activities of some disinfectants and antiseptics by slime layered and mucoid *Staphylococcus aureus*. It was concluded from the studies that these protective coats were responsible for the protective action against the chemical

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Table 1:   The descriptive analysis of the antimicrobial agents

Name	Composition	Manufacturer	Recommended Dilution	Dilution used
Dettol	Chloroxylenol, 4.8%; Oleum pini Aromaticum, 9.0%; Denatured spirits, 11.3%; Sapo vegetalis, 5.8%	Reckitt and Collman, South Africa	1/40-1/20	1/30
Septol	Pine oils, 2.3%; and 5-chloro-2-hydroxydiphenyl methane	Gongoni Ltd., Kano, Nigeria	1/200	1/100
Z germicide	Tar, 7%; and phenol, 2%	Gongoni Ltd., Kano, Nigeria	1/500-1/50	1/100
7 0% ethanol	70% ethanol	BDH Lab. Supplies, England	Stock	Stock

Table 2: Strain source and percentage inhibitory activity of antimicrobial agents on *P. aeruginosa*
Range (mean) of *% inhibition by:

Strain	Source	No of Isolates	Dettol	Septol	Z germicide	70% ethanol
Clinical	Pus	4	94.0-99.7 (96.9)	92.0-99.5 (95.5)	96.0-98.0 (97.0)	99.5-99.9 (99.7)
	Urine	4	94.8-99.6 (97.0)	94.8-99.2 (97.0)	96.0-98.0 (97.2)	99.5-99.9 (99.7)
Environmental	Soil	5	99.8-99.9 (99.9)	99.4-99.8 (99.6)	96.0-99.0 (97.5)	99.8-99.9 (99.8)

percentage of organisms killed in 10minutes; each value is the average of two readings

Table 3: Antibiotic sensitivity pattern of the isolates   *Response (no) to antibiotics:

Strain	Source	No of Isolates	Amp	Chl	Clox	Gen	Pen	Str	Tet
Clinical	Pus	4	R(4)	R(4)	R(4)	R(2), MS (1), S(1)	R(4)	R(3), S(1)	R(4)
	Urine	4	R(4)	R(4)	R(4)	R(1), S(3)	R(4)	R(4)	R(4)
Environmental	Soil	5	R(5)	R(5)	R(5)	S(5)	R(5)	S(5)	R(5)

no of strains; R, resistant, (0-10mm); MS, moderately sensitive, (11-15mm); S, sensitive (>16mm), each is average of two readings; antibiotics are as defined under materials & methods.

agents. However, in this investigation, such protective inhibition was not noticed, and in fact no discriminatory reaction to the chemical agents was noticed among the soil and the clinical isolates that were tested. It can therefore be concluded that these isolates do not possess any protective coat to inhibit the activities of the chemical agents. Since these organisms were isolated from different sources (soil: different part of the country; clinical: different patients), it is believed that required achievement could be made in the programmes of disinfection and antisepsis in Nigeria with regard to *Pseudomonas aeruginosa*.

The results of the antibiotic sensitivity pattern of the isolates as shown in Table 2 indicate that all the strains were resistant. The studies have shown their resistance to broad spectrum antibiotics such as Cloxacillin, Ampicillin, Chloramphenicol and Tetracycline. The relatively high level of resistance to antimicrobial agents is a reflection of misuse or abuse of these agents in the environment (Umoh *et al.*, 1990). About 23% of the isolates were resistant to Gentamicin, while 54% showed resistance to Streptomycin. In all, 23% of the isolates were resistant to all the antibiotics. The effectiveness of the antibiotics is in the order Gentamicin>Streptomycin.

These observations lay credence to the overwhelming reports on the resistance and notoriety of *Pseudomonas aeruginosa* (Duguid *et al.*, 1978; Prescott *et al.*, 1999). Resistance mechanisms in *Pseudomonas* are due to

the possession of R plasmids and multidrug resistance pumps (Prescott *et al.*, 1999). Meanwhile, there seems to be a perfect correlation between the sources of the isolates and their sensitivity pattern to the antibiotics. For instance, all the soil isolates were susceptible to Gentamicin and Streptomicin when compared with the clinical isolates (Table 3). However, there is no perfect correlation between the source of isolation of clinical isolates and the sensitivity pattern to the antibiotics. Amongst the pus isolates, two were resistant to all the antibiotics, one was susceptible to both Gentamicin and Streptomycin, and the remaining isolate was susceptible to Gentamcin only. A similar trend was noticed among the urine isolates (Table 3). Hsu *et al.* (1992) earlier pointed out that differences in the extent of bacterial resistance to various antibiotics may reflect the history of antibiotic application. and may allow drug resistance to be used as an indicator of antibiotic use pattern. In Nigeria, the wide spread distribution of resistant organisms could be a direct consequence of unregulated administration of antibiotics. In many cases, the populace engages in self medication, while circulation of sub-standard, adulterated and expired drugs are rampant.

Among the soil isolates, although the zones of isolation differ, the interpretation of the ambiogram (Chortyk *et a'*, 1993) indicates good susceptibility to Gentamicin and Streptomycin. Thus, it can be deduced that soil inhabiting *Pseudomonas aeruginosa* were less resistant to the antibiotics compared with their clinical partners. The difference in the sensitivity pattern between the strains could probably be due to previous contact of clinical isolates with antibiotics (Kolawole, 1984), and the indiscriminate use of antibiotics (Silva and Hoffer, 1993; Malik and Ahmad, 1994).

However, the extent of resistance shown by all the isolates is a serious concern because in recent times, drug resistance syndrome occurs frequently in isolates obtained from the environment including foods (Manie *et al.*, 1999; Kawakami *et al.*, 2000; Khan and Malik, 2001). Information as obtained in this study on the prevalence of resistance to specific drugs is known to be necessary to understand the magnitude of the problem and to establish baselines for taking action (Caprioli *et al.*, 2000).

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