DRUG RESISTANCE STUDIES ON SOME SELECTED BACTERIA/CLINICAL ISOLATES

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ABSTRACT

Selected pathogenic bacteria obtained from the Noguchi Memorial Institute for Medical Research and the Medical School of the University of Ghana were screened for the presence of the antibiotic resistance gene marker. All the bacteria, except three, were found to be highly resistant to the marker antibiotics used: Salmonella Group D was relatively more sensitive to all three antibiotics (2.0 ug/ml, 10.0 ug/ml and 5.0 ug/ml for Tetracycline, Benzylpenicillin and Streptomycin respectively); Staphylococcus aureus was sensitive to Benzylpenicillin (4.0 ug/ml) and Salmonella typhi was sensitive to Tetracycline (2.0 ug/ml).

The results obtained indicate a high incidence of the antibiotic resistance gene marker among the test bacteria.

Keywords: Drug resistance, gene marker, antibiotics, Bacteria.

INTRODUCTION

The discovery of the efficacy of antibacterial agents in the treatment of the "mirage" of bacterial infections was seemingly an answer to a problem of grave concern to both researchers and clinicians. But the effective use of these "miracle" drugs was short lived due to the emergence of drug resistance or tolerance by target cells/organisms.

This phenomenon of drug resistance is of considerable economic importance and often has grave consequences to the developing world. It also serves as a major challenge to the pharmaceutical industry because the development of resistance ensures that effective drugs are limited in their usefulness [1].

The current socio-economic situation caused by poverty and underdevelopment in developing countries leads to conditions of overcrowding and unhygienic environs thus creating a favourable environment for the interaction of many bacteria leading to the spread of genes among them. These factors coupled with the extensive administration of antibiotics in human and veterinary medicine and their use as supplement in animal feeds have led to the rapid spread of antibiotic resistance genes among bacteria. Such conditions constitute a powerful selective force for the evolution of virulence factors (e.g. resistance factors) which are not only retained but also sorted out and transferred via delivery systems such as plasmids, transposons and bacteriophages (Phages) [2].

Bacterial cells employ a host of mechanisms in resisting drug action, some of which have been outlined above, but the final result - emergence of resistant strains occurs by an interplay of several mechanisms involving many genes. For example, amongst the B-lactams, the mechanism of drug resistance in gram-negative bacteria is due to a complex interaction involving drug affinity for the target site, the lactamase activity, amount of drug in the periplasmic space, and the number of lethal target sites [3].

Currently the following methods are being employed to enhance drug sensitivity; multidrug therapy, enzyme inhibition and chemical modification of the existing antibiotics [4].

Though new advances have been made to combat the threat of drug resistance, a new development is the emergence of multidrug resistance [3].

The specific aim of the study was:

- the screening of some selected pathogenic bacteria for the presence of the antibiotic resistance gene markers.

MATERIALS AND METHODS

Materials

Bacteria

Two sources of bacteria were used. The first group was obtained from the Bacteriology Unit of the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon. The bacteria were *Pseudomonas aeruginosa (Ps. aeruginosa)*, isolated from pus, *Staphylococcus aureus (S. aureus)* from a wound, *Shigella dysenteriae (Sh. Dysenteriae) Salmonella typhi (S. typhi)* and *Shigella flexneri (Sh. Flexneri)* were isolated from faecal samples.

The other group was obtained from the Microbiology Department of the University of Ghana Medical School. They were *Klebsiella* species *(Kleb. sp.), Escherichia coli* (E. coli), *Proteus species (Pro. sp.), Pseudomonas aeruginosa (Ps. aeruginsosa), Shigella flexneri) and Salmonella* Group D (*Sal* Group D). The bacteria were kept on agar slants at 4°C.

Media

Nutrient broth and nutrient agar were used for all the microbiological assays. They were obtained from Fluka Chemie AG, CH-9470 Buchs, Switzerland and Difco Laboratories, Detroit, Michigan, USA.

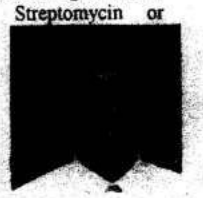
Antibiotics

Streptomycin Sulfate, Benzylpenicillin potassium salt and Tetracycline were used. They were obtained from Fluka Chemie AG, CH-9470, Buchs, Switzerland. A 2mg/ml of antibiotic stock solution was used for all the assays.

All other reagents used were obtained from Fluka Chemie AG, CH-9470, Buchs, Switzerland unless otherwise stated. They were of analytical grade where possible.

Sensitivity Test

The Minimum Inhibitory Concentration (MIC) of the test antibiotics on the bacteria was determined by the agar dilution method. Nutrient Agar plates containing a known amount of antibiotic (Tetracycline, Streptomycin or



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Benzylpenicillin) were prepared. An overnight culture of a selected bacteria strain was diluted in 1% (w/v) nutrient broth. A 1.00ml aliquot of the diluted culture was then added to the agar plates and the cells spread evenly on the plate with a sterile glass spreader. The plates were incubated overnight at 37°C in an Eyela Soft incubator (SL1-600, Tokyo Rikakikai Co., Limited, Japan). For each experiment a control plate without antibiotic was also prepared.

Inhibition or dose response curves were plotted from the data obtained. The MIC's were taken as the lowest antibiotic concentration at which there is no visible growth of bacterial cells during the period of incubation.

RESULTS

SENSITIVITY TEST

The selected pathogenic bacteria were screened for the presence of the antibiotic resistance gene marker by the agar dilution method. Unlike the disc sensitivity tests this method gives a more quantitative reflection of the response of the bacteria to the marker antibiotics. The dose response curves (Figs. 1-3) obtained show a decrease in bacterial cell counts with increasing drug concentration - which reflected the expected trend for an inhibition assay. The MIC's were taken as the lowest antibiotic concentration at which no bacteria growth occurred. These were determined from the curves and recorded as shown in Table 1.

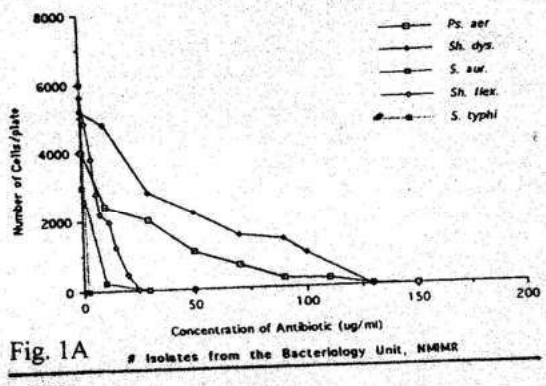

Fig. 1A # Isolates from the Bacteriology Unit, NMIMR

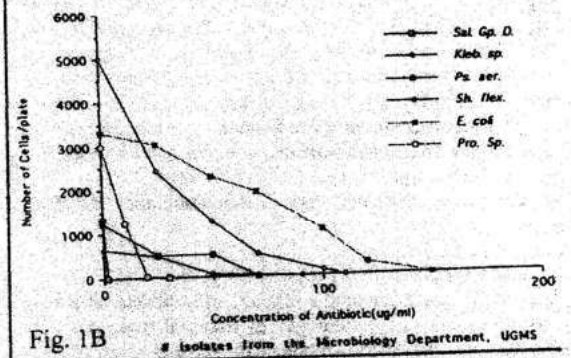

Fig. 1B # Isolates from the Microbiology Department, UGMS

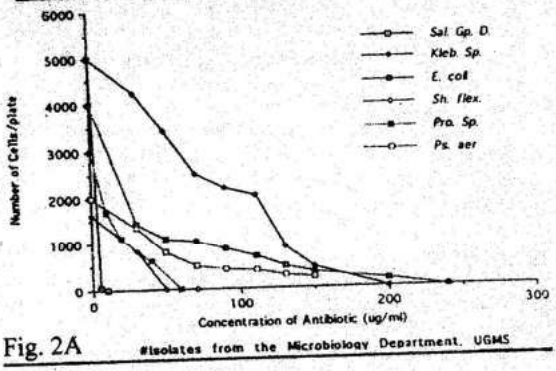

Fig. 2A #Isolates from the Microbiology Department, UGMS

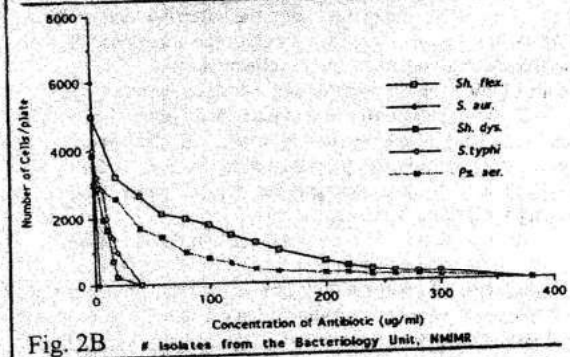

Fig. 2B # Isolates from the Bacteriology Unit, NMIMR

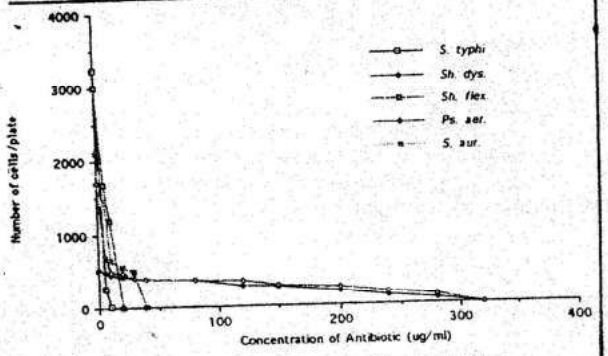

Fig. 3A # Isolates from the Bacteriology Unit, NMIMR

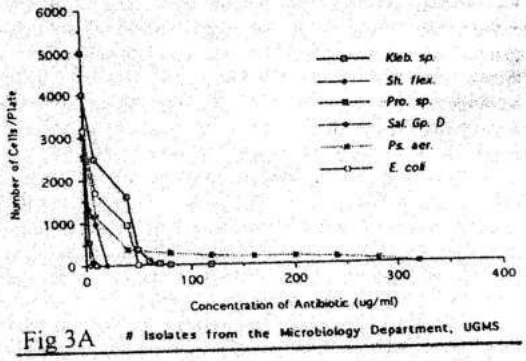

Fig 3A # Isolates from the Microbiology Department, UGMS

Figs. 1-3: Dose response curves of Isolates to the test antibiotics

Table 1: MIC's (ug/ml)

INDICATOR STRAIN	TETRA-CYCLINE	BENZYLPE-NICILLIN	STREPTO-MYCIN
Ps. aeruginosa	135	370	320
Sh. Dysenteriae	130	32	320
S. aureus	34	4	40*
Sh. Flexneri	30	375	20
S. typhi	2	34	20
Sal. Gp. D	2	10	5
Kleb. Sp.	80	172	95
Sh. Flexneri	110	50	20**
E. coli	142	240	50
Pro.sp.	22	60	9
Ps. aeruginosa	68	198	320

*Isolates from the Bacteriology Unit, NMIMR, Legon
** Isolates from the Microbiology Department, UGMS, Legon

All the bacteria showed a high tolerance to the antibiotics except *Sal.* Gp D. which was relatively more sensitive to all the three antibiotics (2 ug/ml, 10 ug/ml and 5 ug/ml for Tetracycline {Tet}, Benzylpenicillin {Ben} and Streptomycin {Str} respectively). *S. aureus* was sensitive to Ben (4 ug/ml) and *S. typhi* to Tet (2 ug/ml). On the average the bacteria from the NMIMR group (refer to materials) were more tolerant to Benzylpenicillin and Streptomycin than the UGMS group. The UGMS group was more tolerant to Tetracycline than the NMIMR group. Irrespective of the source, *PS. aeruginosa* was found to be highly resistant to the marker antibiotics. Ps. aeruginosa and *Sh. flexneri* from the NMIMR group were more tolerant to the marker antibiotic than the UGMS group. Figs. 1-3 show dose response curves for the selected pathogenic bacteria tested for their antibiotic sensitivity by the agar dilution method.

The indicator strains were tested on the antibiotics and their response plotted as in Figs. 1-3. The minimum inhibitory concentration (MICs) was determined as the minimum antibiotic concentration at which the bacteria do not grow. The Table 1 shows the MICs obtained.

DISCUSSION AND CONCLUSION

Bacteria respond in diverse ways to antibiotic administration. Their ability to be susceptible or tolerant to antibiotics is dependent on a complex interplay of factors such as permeability barriers, lack of affinity between the drug and its protein receptor, a suitable intracellular target, the ability to switch to alternative metabolic pathways and the production of detoxifying or hydrolytic enzymes [3]. The response shown by the sensitivity test may be due to any or an interplay of the above factors. Changes in antibiotic susceptibility among different species are a function of the interaction of bacteria genomes (Chromosomes, plasmids and transposons) with specific host and environmental factors [5].

Results from Figs. 1-3 and Table 1 indicate the presence of the marker genes for Tet., Ben. and Str. among the test bacteria. The MICs obtained were found to be higher than that of Bryant [6]. The determined MIC values (Table 1) were about 95% higher than that of Bryant [6] for *S. aureus, E. coli* and *Ps. aeruginosa* for all three test antibiotics. This may be attributed to improper drug administration by clinicians and individuals, introduction of new and well adapted species through infection, crossinfection through vertebrae animals due to the extensive administration of antibiotics as feed supplements, overcrowding and other environmental changes which lead to the selection of resistant mutants. Other factors, which may account for the high MICs obtained are the effect of inoculum size, pH and type of media used. The source of organisms seems to also have an effect on the high MICs recorded. The bacteria from the NMIMR group seem on the average to be more tolerant than the UGMS group. This may probably be due to the fact that the NMIMR being a referral and research centre, the highly resistant isolates of interest were stored for further work and as a bacteria bank for researchers while that of the UGMS group were kept purely for microbiological work without any bias for resistant species. Such cases of high resistance may have arisen due to prolonged exposure to antibiotics. *Ps. aeruginosa* showed a higher tolerance to all the test drugs. This confirms Duerden *et. al.'s* [4] assertion of the development of resistance to virtually all known antibiotics by *Ps. aeruginosa. Sal.* Gp.D. was relatively more sensitive to all three antibiotics, *S. aureus* was sensitive to Benzylpenicillin and *S. typhi* to Tetracycline. The sensitivity shown by these organisms may be due to the presentation of a less formidable resistant mechanism to surmount the drug action [1].

From the discussion it is therefore recommended that infectious disease states be kept under surveillance so as to have an up to date antibiogram. This will help formulate antibiotic policies for hospitals without good laboratory facilities. This knowledge would also be of help in the periodic formulation of the essential drug list. The culture of public education on drug abuse and improper administration must be sustained. A multidisciplinary approach to resistance studies must be stressed so as to evolve a more efficient and rationale drug administration in the country.

ACKNOWLEDGEMENTS

My sincere appreciation to all my former teachers for an exposure to research training and Prof. F.N. Gyang of the Department of Biochemistry for his constructive criticism of the manuscript. A big thank you to Mr. K.Y. Fosu and Maxwell Ofosuhene for allowing me to use their computer facilities.

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