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Comparative in-vitro antibacterial activity of two brands of antibiotics against clinical isolates of some bacterial genera

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Summary

Six brands of ampicillin and four of gentamicin were compared for their in-vitro antibacterial activity against clinical isolates of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The minimum inhibitory concentrations obtained for each brand against each bacterial isolate compared very well with one another, and the kinetics of bactericidal activity showed that the brands of each antibiotic possessed similar activity against the clinical isolates tested.

Résumé

Six genres d'ampicilline et quatre de gentamicine ont été comparés quant à leur activité in-vitro antibactériel contre les isolés cliniques de *Escherichia coli*, *Staphylococcus aureus* et *Pseudomonas aeruginosa*. Les concentrations inhibitoires minimales obtenues pour chaque genre contre chaque isolé bacteriale sont comparables les uns contre les autres et la kinesthésie de l'activité bactéricidale a montré que les genres de chaque antibiotique possédaient presque les mêmes activités contre les isolés cliniques examinés.

Introduction

Evaluation of antibiotics has continued to attract attention on two aspects; one is the determination of in-vitro antibacterial activity and the other is the concentration of antibiotics reached in the blood following a given dose. This study utilizes in-vitro antibacterial activity to investigate the antibacterial potency of the brands of some antibiotics marketed in Nigeria.

Several comparative in-vitro activity studies of many antibiotics have been reported by workers from different parts of the world. Mouton and de Kok Broeren [1] compared the activity of netilmicin with four other aminoglycosides; gentamicin, tobramycin, amikacin and kanamycin, against strains of Gram-negative bacteria and *Staphylococcus aureus*. They found that *Escherichia coli* and *Klebsiella* spp. resistant to gentamicin and tobramycin were sensitive to netilmicin and amikacin, while *Proteus mirabilis* was found sensitive to kanamycin. There were no differences in the speed of killing observed when the five antibiotics were compared for their bactericidal activity. When Watts and Phillips [2] compared the in-vitro activity of 15 penicillins and mecillinam against *Neisseria gonorrhoeae* they found ampicillin and amoxycillin to have shown greater activity against relatively resistant strains of *N. gonorrhoeae* than benzyl penicillin.

We report here the comparative antibacterial activity of brands of ampicillin against strains of *E. coli* and *S. aureus* and brands of gentamicin against strains of *Pseudomonas aeruginosa* in order to ascertain the antibacterial quality of all brands of the two antibiotics available in most registered pharmacy shops in Nigeria at the time of this study.

Materials and methods

Organisms

The bacterial isolates employed in this study consisted of three strains of *E. coli*, four of

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S. aureus and 10 of *P. aeruginosa*, all of which were maintained in the laboratory on agar slopes.

Media

Nutrient broth No. 2 (Oxoid) pH 7.4 and nutrient agar (Oxoid) pH 7.4 were used in this study.

Antibiotics

Six brands of ampicillin sodium powder for injection were obtained from the following sources; Beecham Research Laboratories, Brentford, U.K.; Sanders-Probel SA, Brussels, Belgium; RajRab, West Berlin, FRG; Rotexmedica GmbH, Hamburg, FRG; Heinz-Haupt, Berlin, FRG; and Lab. Oftalmiso, Beniajan Murcia, Spain; and four brands of gentamicin sulphate solution for injection from Eupharma Laboratory, Bombay, India; Lek Ijublijana, Yugoslavia; Medisca, Milan, Italy and MCA, Milan, Italy. Each vial of ampicillin injection contained either 250 or 500 mg of ampicillin sodium while each brand of gentamicin solution for injection contained 40 mg/ml of gentamicin sulphate. The contents of four ampoules of each brand of antibiotic were pooled together for each determination. The brands of the two antibiotics were purchased from registered pharmacy shops in Ibadan. All brands of antibiotics employed in this study were stored at 4°C until needed.

Determination of MIC

The minimum inhibitory concentration of each brand of antibiotics against each bacterial isolate was determined by the broth dilution method of Waterworth [3]. Serial twofold dilutions of each brand of antibiotics were carried out in 5 ml sterile nutrient broth to which was added appropriate bacterial culture, to produce a suspension of 10^6 colony forming units (c.f.u.) per ml. Inoculated tubes were then incubated at 37°C for 48 h and observed for growth. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic required to produce no discernible growth after 48 h of incubation at 37°C. The average MIC was obtained after three determinations were carried out on each brand.

Kinetics of bactericidal activity

National collection type cultures (NCTC) of *E. coli*, *S. aureus* and *P. aeruginosa* were employed to assess the kinetics of bactericidal activity of the antibiotics. A 0.1 ml volume of a diluted culture of each test strain was added to a universal bottle containing 9.9 ml of 0.1% (w/v) peptone:water to produce a bacterial suspension of 10^6 organisms per ml. An appropriate concentration of each brand of antibiotics was added and viable counts were performed at 0, 0.5, 1, 2 and 4 h using the pour-plate method. This method involved making 10-fold serial dilutions of samples taken at different time intervals in sterile distilled water and inoculating 1 ml of a 10^{-4} dilution into a sterile Petri dish into which melted agar, cooled to 45°C, was added. The dilution of the samples served to reduce the activity of the antibiotics to a minimum. The inoculated agar plates were incubated at 37°C for 48 h after which the bacterial colonies were counted. The number of surviving organisms per ml were calculated and the kinetics of bactericidal activity of the brands of antibiotics plotted on a semi-logarithmic graph. The test was repeated to ensure reproducibility.

Assay of ampicillin

Each brand of ampicillin sodium was assayed by the method of the British Pharmacopoeia 1980 [4] to determine the percentage of ampicillin calculated with reference to the anhydrous substance.

Results

The minimum inhibitory concentrations of the different brands of ampicillin against strains of *E. coli* and *S. aureus* are shown in Table 1. The MIC values for the three strains of *E. coli* varied from 1.25 to 5 μg/ml while the values obtained for the four strains of *S. aureus* ranged from 0.15 to 0.6 μg/ml. The MIC values of 1 or 2 μg/ml obtained for the four brands

Table 1. The minimum inhibitory concentration (MIC) of each brand of ampicillin injection against strains of *E. coli* and *S. aureus*

Strain of bacterial species	MIC of particular brand for each isolate (μg/ml)					
	Beecham (90%)*	Rotexmedica (107%)*	Sanders (100%)*	Heinz-Haupt (111%)*	RajRab (98%)*	Lab. Oftalmiso (95%)*
E. coli						
NCTC 14RS	5.00	5.00	5.00	2.50	5.00	5.00
Strain K12	2.50	1.25	2.50	1.25	1.25	2.50
Strain 7	1.25	2.50	1.25	1.25	2.50	2.50
S. aureus						
NCTC 6571	0.30	0.30	0.30	0.30	0.60	0.30
PHM 2287	0.30	0.60	0.30	0.30	0.30	0.30
PHM 3287	0.30	0.30	0.15	0.15	0.15	0.30
PHM 1287	0.15	0.30	0.15	0.30	0.30	0.30

*Percentage of ampicillin in each brand of ampicillin sodium with reference to the anhydrous substance.

Table 2. The minimum inhibitory concentration (MIC) of each brand of gentamicin injection against strains of *P. aeruginosa*

Strains of the organism	MIC of particular brand for each isolate (μg/ml)			
	Ijublijana	Eupharma	Medisca	MCA
NCTC 6750	1	1	1	1
UCH 18995	2	2	2	1
UCH 19475	1	1	2	1
UCH 19286	2	1	2	1
UCH 16803	1	1	1	2
UCH 19276	2	1	2	2
UCH 17723	> 16	> 16	> 16	> 16
UCH 17567	1	2	1	1
UCH 18835	1	2	1	2
UCH 17818	1	2	1	2

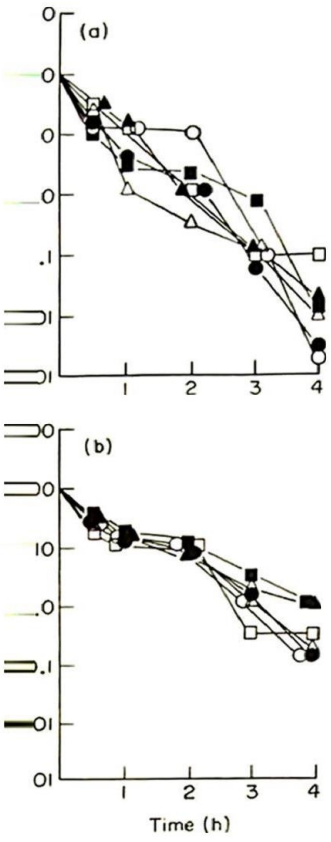
of gentamicin against the 10 strains of *P. aeruginosa* are shown in Table 2 where strain UCH 17723 was found to be resistant to all brands of gentamicin tested. The percentage of ampicillin obtained from assaying each brand of ampicillin sodium is shown in parentheses in Table 1.

The results obtained for the kinetics of bactericidal activity are shown in Figs 1 and 2. Using 5 μg/ml of each brand of ampicillin sodium against *E. coli* reduced the bacterial population to between 0.003% and 0.1%, while with 0.3 μg/ml of ampicillin the population of *S. aureus* was reduced to between 0.1% and 1% after 4 h contact with the ampicillin sodium injection. The percentage of *P. aeruginosa* inoculum that survived 4 h contact with 1 μg/ml of the four brands of gentamicin sulphate was between 0.3 and 0.7 (Fig. 2).

Discussion

This study has shown that the antibacterial activity of each brand of ampicillin sodium against strains of *E. coli* and *S. aureus* compared very favourably with one another, and none of the six brands tested could be said to be significantly different from the others.

However, it was observed in the kinetics of bactericidal activity of the brands of ampicillin sodium against *E. coli*, Penbritin produced the highest percentage of survivors, although the percentage survival was not significantly different from the percentage of survivors produced by other brands. This could be due to the fact that Penbritin contained the lowest percentage of ampicillin in our assay study. The situation in respect of *S. aureus* was different, and consequently little emphasis must be



— of bactericidal activity of (a) 5 μg/ml
—/ml of (□) Beecham, (○) Sanders-
—cinz-Haupt, (●) RajRab, (■) Rotex-
—▲) Oftalmiso brands of ampicillin
E. coli and *S. aureus* respectively.

— percentage of ampicillin present
d of ampicillin since BP (1980)
—nded that ampicillin sodium
—n not less than 87% of ampicil-
Penbritin brand contained more
—imum.
—on with the brands of gentamicin
similar to that of ampicillin. The
—activity of the four brands against
—en isolates of *P. aeruginosa* was
—cal.
—ance of the results obtained in this
the fact that the brands of the two
—vestigated came from different
—as such it is of interest to observe
in antibacterial activity of these
tibiotics. Our findings agreed in
—findings of Ogunbona and Akanni

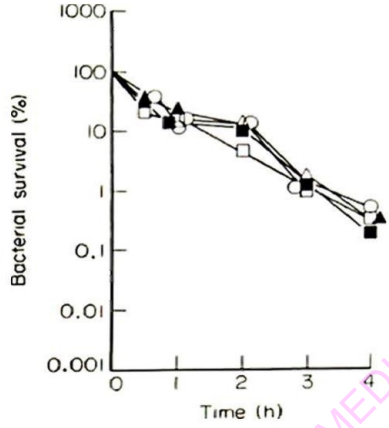


Fig. 2. Kinetics of bactericidal activity of 1 μg/ml of (□) Eupharma Lab, (△) Lek Ijublijana, (○) MCA and (■) Medisca brands of gentamicin sulphate against *P. aeruginosa*.

[5] in their comparative bioavailability studies on some brands of ampicillin capsules. It is pertinent to mention that comparative studies of this nature should be a continuous exercise, with the sole aim of detecting fake drugs which may be in circulation in some of our retail pharmacy outlets in Nigeria today. Reports of fake ampicillin capsules appears to be rampant, but at the time of our study all brands of ampicillin and gentamicin tested, possessed potent antibacterial activity against all clinical isolates.

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