The African Journal of Medicine and Medical Sciences

Volume 13
1984

BLACKWELL SCIENTIFIC PUBLICATIONS
Oxford London Edinburgh Boston Palo Alto Melbourne

Afr. J. Med. med. Sci. (1984) **13**, 15–20.

EFFECTS OF SALIVA AND ALPHA-AMYLASE ON ANTIBIOTIC SENSITIVITY OF BACTERIA

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Summary

Two hundred and ninety-six bacterial isolates were investigated for the effects of saliva and alpha-amylase on their susceptibility to ampicillin, tetracycline, chloramphenicol and gentamicin. When the test organisms were primed with normal and 'diseased' saliva there were no observable differences in the MICs of ampicillin and chloramphenicol for group-A streptococci, but alpha-amylase significantly reduced the MIC of tetracycline from 2 to 0.25 mg/l. With *Staphyloccus aureus*, priming with saliva and alpha-amylase had no effect on the MICs of gentamicin and ampicillin, whereas the MICs of tetracycline and chloramphenicol were increased. The effect of saliva on the susceptibility of *E. coli* to tetracycline was also significant; MIC_{50} and MIC_{90} were reduced from 128 to 8 and 32 mg/l respectively. Chloramphenicol was however increased from less than 0.125 to 1 and 2 mg/l when *E. coli* was primed with amylase and saliva respectively. The general significance of these observations is discussed.

Résumé

On a recherché chez 296 bacterie isolée pour les effets du salive et alpha-amylase sur les sensibilités a ampicillin, tétracyline, chloramphenicol et gentamycine. Quand les organismes sur teste ont étaient amorcer avec la salive normale et infectée, il n'avait pas les différences observable dans les MICs d'ampicillin et chloramphenicol pour groupe-A streptococci, mais alpha-amylase avait reduite significativement le MIC de tétracyline de 2 a 0.25 mg/l. La salive et alpha-amylase dans *staphylococcus aureus* n'avaient aucune effet sur les MICs de gentamycine et ampicillin, au lieu que les MICs de tetracycline et chloramphenicol étaient augmenter l'effect du salive sur le sensibilité de *E. coli* à tétracycline était significatif; MIC_{50} et MIC_{90} étaient reduite de 128 à 8 et 32 mg/l respectivement. Chloramphenicol etait cependant augmenter de moins de 0.125 à 1 et 2 mg/l quand *E. coli* était amorcer avec alpha-amylase et du salive respectivement. Le significance generale de ces observations est discuté.

Introduction

Saliva has been shown to have antibacterial properties, and these properties have been attributed to substances present in it, e.g. lysozymes (Salton, 1967), α-amylase (Mellersh, Clark & Hafiz, 1979) and lacto-peroxidase (Steele & Morrison, 1969). The suggestion was that these substances kill bacteria by acting on different cell-wall components of susceptible Gram-positive and Gram-negative bacteria; these two groups of bacteria are sensitive to different types and concentrations of antibiotic. The differences in sensitivity to antibiotics are due mainly to permeability of the cell wall.

Kareem and Arain (1981) and Kareem, Arain and Oluani (1982) in their studies reported that enzyme activity in normal human saliva differs from the activity in saliva present in mouth with pathological lesions. This study was undertaken to compare the effect of α-amylase and of saliva obtained

from normal healthy mouth with that from the mouth with infective lesions, on bacterial susceptibility to antibiotics.

Materials and methods

Test organisms

A total of 296 bacterial strains including three reference strains of *Escherichia coli*, NCTC 10418, *Staphylococcus aureus* NCTC 10662 were used. The other strains of bacteria used are as follows: *E. coli* (fifty-two strains), Beta-hemolytic streptococci Group A (sixty-eight strains), *Staph. aureus* (fifty), *Pseudomonas aeruginosa* (fifty-four), *Proteus mirabilis* (twenty-two), *Shigella boydii* (thirty-two) and *Klebsiella* spp. (nineteen). These organisms were isolated from a variety of clinical specimens examined in the bacteriology laboratory of the Lagos University Teaching Hospital. Final identification was done by standard method.

Media

The media used were: Mueller–Hinton agar (oxoid), Mueller–Hinton broth (oxoid), MacConkey agar (oxoid), Blood agar (blood agar base No. 2 (oxoid) plus 7.5% human blood), lysed horse blood (oxoid), Todd Hewitt broth.

Antibiotics

The following antibiotics were used and kindly supplied as follows: Chloramphenicol (Parke Davis & Co., Pontypool, Gwent, U.K.), tetracycline (G. Cyanamid of Gt Britain Ltd, London), ampicillin (Beecham Res. Lab. Brentford, U.K.) and gentamicin.

Collection and treatment of saliva

Saliva specimens were collected from normal healthy volunteers working within our institution. All of these subjects were free from dental caries and active lesions or past history of dental problems. About 20 ml of saliva was collected in pre-sterilized plastic universal containers (Sterilin) from each subject within 15–30 min after chewing dental wax which stimulated rapid flow of saliva. Another 20 ml of saliva was collected from adult patients with infective lesions in the mouth, who attended the dental clinic of our hospital for the treatment of periodontal and carious lesions.

All specimens of saliva collected were centrifuged at 2000 × *g* for 15 min and the supernatant carefully collected and stored at 4°C.

Reconstitution of α-amylase

Solution of α-amylase was made by dissolving 0.1 g of commercial amylase powder (BDH, laboratories) in 10 ml of Mueller–Hinton broth (oxoid). The amylase solution was then sterilized by passing through seitz filters (oxoid).

Pre-susceptibility 'priming'

For the 'priming' of the bacteria the following media were prepared: (i) Mueller–Hinton broth dispensed in 5 ml amounts into bijoux bottles and filter sterilized; (ii) 20 ml of sterile Mueller–Hinton broth plus 20 ml of sterile saliva; the mixture was sterilized by filtration and dispensed in 5 ml amounts into a second set of bijoux bottles; and (iii) 100 ml of Mueller–Hinton broth to which 1 g of α-amylase had been added was dispensed into another set of bijoux bottles and also sterilized by filtration. Each bottle was then seeded with approximately 10^4 colony-forming units/ml (CFU/ml) of the test organisms and incubated in air at 37°C for 18 h. Todd–Hewitt broth was substituted for Mueller–Hinton broth when streptococci were tested.

In this study, each bacterial strain was primed five times with saliva from five different normal mouths, four times with saliva from four mouths with pathological lesions; minimum inhibitory concentrations (MICs) were determined for each organism after 'priming' with these saliva samples and the average MICs recorded.

Susceptibility test

Minimum inhibitory concentrations of ampicillin, chloramphenicol, gentamicin and tetra-

cycline were performed by the agar dilution technique. Serial doubling dilutions of the antibiotics were made in sterile water; 2 ml of each dilution was then added to 18 ml of Mueller–Hinton agar to give final concentrations of 0.125 to 128 mg/l. Five percent lysed horse blood (oxoid) was added to the media when experimenting with streptococci. The plates were then dried at 37°C ready for use. An 18-h culture of each 'primed' bacteria was diluted in sterile water to give an inoculum of approximately 10^4 CFU/ml; 0.1 ml of each bacterial culture was then seeded onto each plate containing appropriate concentrations of the test antibiotics. All plates were then incubated in air at 37°C for 24 h. Standard strain of Oxford *Staph. aureus* (NCTC 6571) and *E. coli* (NCTC 10418) were included as appropriate in each batch as control. Sensitivity agar plates were freshly prepared daily for the test. The MICs of the antibiotics were taken as the lowest concentrations which completely inhibited the growth of each strain of the bacteria after 24 h incubation in air.

Results

A total of 296 bacterial isolates were used (see Table 1), nineteen strains of *Klebsiella spp.* also tested are not included in the table (because their MICs were >128 mg/l).

The MICs for group-A streptococci of ampicillin, tetracycline, gentamicin and chloramphenicol are shown in Table 2. Streptococcal strains had MIC of ampicillin at less than 0.125 mg/l without priming with amylase or saliva (referred to as normal MIC) and these values were maintained after 'priming' with

TABLE 1. Source and number of organisms tested

Bacteria	Number	Source
Strept. pyogenes (Gp A)	68	Sore throat
Staph. aureus	55	Soft tissue infections
E. coli	55	Urinary-tract infections (UTI)
Ps. aeruginosa	54	Burns
P. mirabilis	52	UTI
Sh. boydii	23	Gastroenteritis

TABLE 2. MICs of ampicillin, tetracycline, gentamicin and chloramphenicol for 'primed' group-A streptococci

Antibiotics	Organisms* 'primed' with:	No. of strains	MIC (mg/l) Range	50%	90%
Ampicillin	'Normal'	68	0.125	0.125	0.125
	Amylase	68	0.125	0.125	0.125
	Saliva	68	0.125	0.125	0.125
	Infected saliva	68	0.125	0.125	0.125
Tetracycline	'Normal'	68	1–2	1	2
	Amylase	68	0.25–2	0.25	0.25
	Saliva	68	0.125–16	0.125	2
	Infected saliva	68	0.125–2	0.125	2
Gentamicin	'Normal'	68	2–4	4	4
	Amylase	68	2	2	2
	Saliva	68	1–2	1	2
	Infected saliva	68	1–2	1	2
Chloramphenicol	'Normal'	68	2–4	4	4
	Amylase	68	2	2	2
	Saliva	68	1–8	2	4
	Infected saliva	68	2–8	2	4

*'Normal': normal MIC of test organism. Amylase: test organism was 'primed' with α-amylase before MIC was determined. Saliva: test organism was 'primed' with saliva from normal mouth before MIC was determined. Infected saliva: test organism was 'primed' with saliva from mouth with pathological lesions before MIC was determined.

amylase and saliva. The MIC range of tetracycline was between 1 and 2 mg/l; 1 mg/l inhibited 50% and 2 mg/l inhibited 90% of the strains. MIC_{50} and MIC_{90} of streptococci for tetracycline decreased to 0.25 mg/l when 'primed' with amylase. When primed with saliva the MIC_{50} decreased to less than 0.125 mg/l. The normal MIC of gentamicin for 50 and 90% of the organism was 4 mg/l. Priming of streptococci with saliva resulted in a decrease in the MIC_{50} of the strains from 4 to 1 mg/l for gentamicin. The normal MIC of chloramphenicol for streptococci was between 2 and 4 mg/l; MIC_{50} and MIC_{90} was 4 mg/l.

The MICs of the test antibiotics for *Staph. aureus* are shown in Table 3. All strains tested were β-lactamase negative. Normal MIC of ampicillin was between 0.25 and 1.0 mg/l. Fifty percent of the strains were inhibited at concentration of 0.25 mg/l. No changes were observed when *Staph. aureus* was primed with amylase or saliva. The normal MIC of tetracycline for *Staph. aureus* was between 0.125 and 2 mg/l. When primed with amylase and saliva, the MIC was 16 and 8 mg/l respectively. For chloramphenicol the MIC for *Staph. aureus* was less than 0.125 mg/l for 90% of the strains. However, when *Staph. aureus* was primed with amylase and saliva from mouth with lesions, MIC_{90} of chloramphenicol increased to 2 mg/l. The normal MIC of gentamicin for *Staph. aureus* was less than 0.125 mg/l. There were no observable changes in sensitivity when primed with amylase and saliva.

The normal MIC for *E. coli* of ampicillin was between 1 and 128 mg/l (see Table 4); MIC_{50} and MIC_{90} were greater than 128 mg/l, when primed with amylase or saliva. For the same organism the normal MIC_{90} and MIC_{50} of tetracycline were 128 mg/l each but decreased to 32 and 8 mg/l respectively when primed with saliva from normal mouth. Chloramphenicol had a normal MIC for *E. coli* at a concentration less than 0.125 mg/l and this value was increased to 1 mg/l each for 50 and 90% of the strains when primed with amylase and normal saliva. There was also an increase of MIC_{90} to 2 mg/l when primed with saliva from diseased mouth.

Results obtained for *Sh. boydii*, *Ps. aeruginosa* and *P. mirabilis* were not included in the tables; however, these organisms also demonstrated changes in sensitivity when 'primed' with amylase or saliva. *Ps. aeru-*

TABLE 3. MICs of ampicillin, tetracycline, gentamicin and chloramphenicol for 'primed' *Staph. aureus*

Antibiotics	Organisms* 'primed' with:	No. of Strains	MIC (mg/l) Range	50%	90%
Ampicillin	'Normal'	55	0.25–1.0	0.25	1
	Amylase	55	0.25–1.0	0.25	1
	Saliva	55	0.25–1.0	0.25	1
	Infected saliva	55	0.25–1.0	0.25	1
Tetracyline	'Normal'	55	0.125–2	0.125	2
	Amylase	55	0.125–16	0.125	16
	Saliva	55	0.125–8	0.125	8
	Infected saliva	55	0.125–8	0.125	8
Chloramphenicol	'Normal'	55	0.125	0.125	0.125
	Amylase	55	1–2	2	2
	Saliva	55	0.5–1	1	1
	Infected saliva	55	0.5–2	2	2
Gentamicin	'Normal'	55	0.125	0.125	0.125
	Amylase	55	0.125	0.125	0.125
	Saliva	55	0.125	0.125	0.125
	Infected saliva	55	0.125	0.125	0.125

*See footnote to Table 2.

TABLE 4. MICs of ampicillin, tetracycline, gentamicin and chloramphenicol for 'primed' *E. coli*

Antibiotic	Organism* 'primed' with:	No. of Strains	MIC (mg/l) Range	50%	90%
Ampicillin	'Normal'	55	1–128	128	128
	Amylase	55	4–128	128	128
	Saliva	55	2–128	128	128
	Infected saliva	55	2–128	128	128
Tetracycline	'Normal'	55	1–128	128	128
	Amylase	55	2–128	128	128
	Saliva	55	2–32	8	32
	Infected saliva	55	2–32	16	32
Chloramphenicol	'Normal'	55	0.125	0.125	0.125
	Amylase	55	1	1	1
	Saliva	55	0.5–1	1	1
	Infected saliva	55	1–2	1	2
Gentamicin	'Normal'	55	0.125	0.125	0.125
	Amylase	55	0.125	0.125	0.125
	Saliva	55	0.125	0.125	0.125
	Infected saliva	55	0.125	0.125	0.125

*See footnote to Table 2.

ginosa and *Klebsiella spp* were very resistant to ampicillin, tetracycline, chloramphenicol and gentamicin; all had MICs of greater than 128 mg/l.

Discussion

Our data showed that saliva or α-amylase had a variety of effects on the susceptibility of a number of micro-organisms to antimicrobial agents.

There were no observable changes in the sensitivity of gram-positive bacteria to ampicillin, which acts on the cell wall, but there was altered sensitivity to some of the antibiotics that acted intracellularly. All the gram-negative bacteria tested in this study altered their sensitivity to all the antibiotics, in one way or the other (except gentamicin in the case of *E. coli*), after 'priming' with amylase or saliva.

Our data also indicated that gram-positive bacteria were stable to the effect of saliva and amylase whereas the converse was true for the strains that were gram-negative. Except for tetracycline, saliva and amylase have similar and reproducible effects on the sensitivity of all the test organisms to other antibiotics. It was observed that the effects of saliva or amylase on chloramphenicol were generally 'antagonistic' for all the test organisms, while for gentamicin they were 'synergistic'; tetracycline and ampicillin had variable effects on the organisms. This phenomenon suggests that α-amylase is probably the active principle in saliva that is responsible for the changes in bacterial cell wall when treated with saliva. Mellersh *et al.* (1979, 1980) who reported the antigonococcal properties of salivary amylase also stated that gonococci, a gram-negative bacterium, treated with amylase became more resistant to penicillin when compared to non-amylase-treated gonococci.

It is interesting to note that the changes produced on the susceptibility of bacteria by treatment with α-amylase were usually more pronounced than those produced by treatment with saliva. The reason for this may be due to the lower amylase activity in saliva relative to that present in the amylase solution used to 'prime' the test strains.

The results of this study also show that the higher the amylase activity, the more damage is done to the cell wall, and the more its effect on antibiotic sensitivity. Sanderson *et al.* (1974) observed that the greater the lesion on

the lipopolysaccharide (LPS) component of the bacterial cell wall, the greater the effect to the sensitivity of antibiotics. Our data show that gram-positive bacteria did not alter their sensitivity to ampicillin when 'primed' with saliva or amylase, whereas the gram-negative bacteria had variable sensitivity after similar treatments. In about 80% of the strains the effect of saliva and amylase increased the sensitivity of streptococci, *Ps. aeruginosa*, *Sh. boydii* and *Pr. mirabilis* to gentamicin. In contrast, saliva and amylase decreased the sensitivity of *E. coli*, *Staph. aureus* and *Pr. mirabilis* to gentamicin. We also observed from experiments with tetracycline that the MIC for *Pr. mirabilis*, *Sh. boydii*, and *Ps. aeruginosa* was increased by priming with saliva only. However, when *Staph. aureus*, *E. coli*, *Ps. aeruginosa*, *Sh. boydii* and *Pr. mirabilis* were 'primed' with amylase the sensitivity to tetracycline remained unchanged, and in some cases was markedly decreased.

Generally, saliva and amylase increased the sensitivity of streptococci to tetracycline, gentamicin, chloramphenicol and similarly the effect of saliva as well as of amylase on *Pr. mirabilis* increased its sensitivity to ampicillin, tetracycline and gentamicin. There was no significant difference between the effect of normal saliva and saliva obtained from mouth with infective lesions on the sensitivity of the bacterial strains. Both types of saliva demonstrated similar effects on the test organisms, although in a recent report by Kareem and Arain (1981), saliva from mouth with infective lesions was suggested to have greater amylase activity generally than saliva from normal mouth.

The alterations in the sensitivity of bacteria to antibiotics by saliva and α-amylase demonstrated in this study may explain some of the discrepances observed between *in vitro* and *in vivo* sensitivity of bacteria to some particular antibiotics. It is therefore important to correlate the clinical response of individual patients to the sensitivity results emanating from the diagnostic laboratory.

Acknowledgments

We are grateful to our colleagues in the Dental Clinic of the Lagos University Teaching Hospital (LUTH) for their co-operation in obtaining specimens from their patients.

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(*Received* 16 *August* 1983; *accepted* 4 *October* 1983)