

AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 24, NUMBER 2, JUNE 1995



EDITOR: B.O. ONADEKO

ASSISTANT EDITORS:

B.O. OSOTIMEHIN and A.O. UWAIFO



SPECTRUM BOOKS LIMITED
Ibadan • Owerri • Kaduna • Lagos

ISSN 1116-4077

Plasmid profiles and antibiotic susceptibility patterns of *Staphylococcus aureus* isolates from Nigeria

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Summary

In an investigation into the problems of infections due to *Staphylococcus aureus* in Nigeria, 100 strains were isolated from various hospitals in Lagos. The strains were screened for the presence of plasmids and for susceptibility to antimicrobial agents. Plasmids were extracted by modification of the method of Takahashi and Nagono[1]. The plasmids were diverse in nature. The strains were found to be highly resistant to commonly prescribed antibiotics.

Résumé

Au cours d'une étude des problèmes des infections dues au *Staphylococcus aureus* au Nigéria, 100 souches ont été isolées dans des hôpitaux différents à Lagos. Les souches ont été examinées pour déterminer la présence des plasmides et leur sensibilité aux agents anti-microbes. Les plasmides ont été extraits par la méthode de Takahashi et Nagono; les plasmides étaient d'une variété diverse: les souches résistaient aux antibiotiques qui sont habituellement prescrits.

Introduction

Staphylococcus aureus is known to produce a wide spectrum of diseases ranging from superficial skin lesions to serious systemic infections[2]. The epidemic strains are commonly resistant to many antibiotics, and these multi-resistant strains cause nosocomial infections. *S. aureus* strains have been known to be resistant to as many as 20 antimicrobial agents including disinfectants and antiseptics thus making the choice of appropriate therapy difficult[3]. Thus the spread of staphylococcal infections require careful monitoring of isolates by microbiologists. Reliable information about the susceptibility patterns of these pathogens is necessary in order to find solutions to problems created by antibiotic resistance.

Staphylococcus aureus was one of the earliest bacteria species in which extrachromosomal

inheritance was recognised[4]. However very little attention was paid to plasmids occurring in *Staphylococcus*, because of the absence of a rapid, convenient and reproducible method for screening for staphylococcus plasmids. A number of plasmid encoded functions e.g. antibiotic and heavy metals resistance and cryptic plasmids occur in *Staphylococcus*[5]. The wide variety of plasmids present in staphylococci, has made the use of plasmid profiles convenient for studying outbreaks in these bacteria. In addition, the genetic basis of antibiotic resistance in *Staphylococcus* has long been a subject of interest and speculation amongst microbiologists. This work therefore investigates the antibiotic resistance patterns and plasmid DNA profiles of some *S. aureus* isolates in Nigeria.

Materials and methods

Bacteriology

One hundred clinical isolates of coagulase-positive staphylococci, were collected from some medical laboratories and hospitals in Lagos and were characterised using established methods to ascertain and confirm that they were indeed *Staphylococcus aureus* isolates. Such methods include: Colonial morphology, gram stain characteristic, ability to coagulate rabbit serum, ability to produce the enzyme peroxidase, and by the presence of heat-stable DNAase activity.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility patterns of the clinical *S. aureus* isolates were determined by the disc diffusion method of Brown and Blower[6], on Mueller-Hinton agar plates, using the following antibiotics: (Oxoid) 10mcg Ampicillin, 10mcg Chloramphenicol, 5mcg Erythromycin, 10mcg Cloxacillin, 10mcg Gentamicin, 1 unit Penicillin, 10mcg Streptomycin and 10mcg Tetracycline.

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Plasmid Isolation

Initial attempts in our laboratory, to screen for plasmids among our local isolates using various methods, did not give satisfactory results. A modification of the methods of Takahashi and Nagano[1] was found to be effective. Colonies were scrapped from Mueller-Hinton agar (Oxoid), into eppendoff tubes containing a mixture of lysing buffer and lysostaphin or lysozyme in the case of the standard (*E. coli*, V517), and then subsequently incubated, then treated with detergent (2% SDS), alkaline denaturation, chloroform and ethanol precipitation.

Gel electrophoresis was carried out using 0.8% Agarose in Tris borate buffer. Gels were stained with ethidium bromide (0.5 ug/ml) for 45 minutes and observed, and photographed under U-V light.

Escherichia coli (V517), a strain carrying known plasmid molecular weight standards, was provided by Dr. J. Crossa (Oregon Health Sciences University, Portland, Oregon, U.S.A.). Strain V517 contained eight plasmids having the following molecular weights: 55.5, 7.4, 5.7, 5.3, 4.0, 3.1, 2.8, 2.2 kilobases (1.5kb = 1 megadalton). Other *E. coli* K12 carrying plasmids of molecular weights ranging from 52kb to 120kb were from the National Institute for Medical Research collection.

Results

Majority of the strains tested were found to be sensitive to gentamicin, while being resistant to most broad-spectrum antibiotics. Prominent amongst resistance patterns was a basic recurring pattern showing 87% resistance to penicillin. There was also 80% resistance to ampicillin, 82% to erythromycin, 83% to streptomycin, 85% to cloxacillin and 80% to tetracycline. 50% of the isolates were resistant to chloramphenicol and only 30% to gentamicin (Table 1). One the whole, the *S. aureus* isolates showed ten basic resistance patterns (Table 2). No isolate was completely resistant to all the antibiotics used.

Table 1: Susceptibility patterns of 100 *Staphylococcus aureus* isolates from Nigeria

Antibiotics	No and % of resistant isolates	No and % of sensitive isolates
Penicillin	87	13
Chloramphenicol	50	50
Erythromycin	82	18
Gentamicin	30	70
Streptomycin	83	17
Tetracycline	80	20
Cloxacillin	85	15
Ampicillin	80	20

Table 2: Antimicrobial resistance patterns of 100 clinical *Staphylococcus aureus* strains in relation to plasmid contents

S/No	Antimicrobial resistance pattern	% showing resistance pattern	No with plasmids	No with resistance plasmids
1.	Pen	8	5	4
2.	Pen Ery	12	8	5
3.	Pen Ery Str	18	12	10
4.	Pen Str Amp	10	6	3
5.	Pen Chl Clx	25	14	8
6.	Pen Amp Ery Gen	4	2	0
7.	Amp Clx Pen	9	6	2
8.	Clx Ery Pen Tet	5	3	1
9.	Ery Pen Tet Clx Str	6	3	1
10.	Pen Ery Str Tet Chl Clx Amp	7	5	3

Key:

Pen — Penicillin	Amp — Ampicillin
Ery — Erythromycin	Chl — Chloramphenicol
Str — Streptomycin	Clx — Cloxacillin
R — Resistance	Gen — Gentamicin

Table 3: Examples of plasmid — containing *Staphylococcus aureus* isolates and their molecular weights

Isolate number	Plasmid size (Megadaltons)
NM 7	54.5
NM 11	7.5; 3.6
NM 12	77.5; 6.0
NM 18	36; 4.8; 3.0
NM 33	20.0; 15.7; 3.6; 3.0
NM 34	75.0; 65.6; 3.0
NM 40	30.0; 25.7; 10.5
NM 43	5.0; 3.0
NM 51	65.5; 20.0; 4.8
NM 81	20.0; 6.0; 2.5
NM 84	71.0; 5.0
NM 90	10.0; 5.0; 3.5
NM 91	15.0
NM 97	20.0; 10.5; 3.0

Of the 100 isolates screened, 64, contained one or more plasmids of different molecular weights, a few weighing up to 77.5 MD. However most of the plasmids were less than 7MD. Plasmid profiles distinguished more strains than did antimicrobial susceptibility pattern (Table 2). Also plasmid profiles further differentiated isolates with similar antimicrobial susceptibility patterns. One plasmid profile is considered to be different from another, when the difference is in terms of one or more plasmids. Table 3 shows an example of data collected on the plasmid profile of the isolates.

Discussion

The plasmid profile analysis and antimicrobial susceptibility testing was carried out on clinical isolates of *S. aureus* from Nigeria. Although many studies have been conducted on the antimicrobial susceptibility patterns of local isolates of *S. aureus*, there is no report on the plasmid profiles of the local strains of this group of bacteria.

Results of this investigation further confirm that most clinical isolates of *S. aureus* are resistant to a large number of commonly prescribed antimicrobial agents. In a previous study, it was observed over a 2-year period, that the proportion of staphylococcal strains resistant to penicillin, streptomycin and tetracycline, had risen from 15% to 83% amongst

in-patients[7]. Davis[8] also confirm from *in vitro* sensitivity tests that the incidence of resistance of these strains to those drugs, in Lagos represented a serious threat to health. Also *S. aureus* strains isolated from clinical material, mainly pus from various lesions, have been found to show marked resistance to the following antibiotics: penicillin, streptomycin, chloramphenicol and tetracycline, with resistance being more marked in the case of penicillin than the other 3 drugs[9]. Antimicrobial resistance patterns in this study revealed a total of 10 patterns. Olukoya *et al*[10], attributed the widespread resistance observed in developing countries to the indiscriminate use of commonly prescribed antibiotics.

In this work, no attempt has been made to conduct a large survey by plasmid screening. The idea was to compare the antimicrobial resistance patterns and plasmid DNA profiles of isolates within Lagos metropolis. The study has shown that the plasmid contents were diverse in nature. The plasmid profile differentiated very specifically strains among these isolates. These differences may be due to the presence of a large number of circulating strains. Similar to what was found for *Shigella* isolates[11], plasmid profile analysis may be more useful in epidemiological studies in areas of Nigeria with less diverse populations — which is the type of population normally found in Nigerian villages.

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(Accepted 11 September, 1992)

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