International Journal of Medicine and Medical Sciences Vol. 3(5), pp. 149-154, May 2011
Available online http://www.academicjournals.org/ijmms
ISSN 2006-9723 ©2011 Academic Journals

Full Length Research Paper

Staphylococcus aureus isolated from septic caesaerean wound at Ile Ife Nigeria: Antibiotics susceptibility patterns

Adekunle Odunayo Adejuwon[1,3]*, David Ogunkanmbi[3], Mary Adejumoke Bisi–Johnson[3], Bridget Oluwatoyin Fadeyi[2] , Oyinade Aina Agboola[4] and Adebanke Oluwatosin Adejuwon[5]

[1]Department of Biochemistry and Microbiology, Faculty of Information Technology and Applied Sciences, Lead City University, Ibadan, Oyo State, Nigeria.
[2]Department of Nursing, Faculty of Information Technology and Applied Sciences, Lead City University, Ibadan, Oyo State, Nigeria.
[3]Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Oyo State, Nigeria.
[4]Department of Physical and Health Education, Adeyemi College of Education, Obafemi Awolowo University, Ondo State, Nigeria.
[5]Department of Human Nutrition, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.

Accepted 11 March, 2011

Strains of *Staphylococcus aureus* were isolated from a 36-year old woman presented with septic caesaerean wound. The isolates were Gram positive, catalase positive, coagulase positive; heamolysed blood plasma and liquefied gelatin. However, they were methyl red and Voges Proskaur test negative and fermented sugars with acid production. They produced gas only in glucose and lactose. Some of the strains were susceptible to cephalexin, (a first generational cephalosporin), clindamycin (lincosamides), septrin (sulphanamide and trimethoprin), norfloxacin and ciprofloxacin (fluroquinolones) at varying degrees. All the strains were resistant to gentamycin and totally susceptible to drovid. This resistance to antibiotics might have arisen as a result of antibiotics prophylaxis prior to surgery, cross contamination of the wound surface during post surgical wound dressing or from health care personnels.

Key words: *Staphylococcus aureus*, septic caesaerean wound, antibiotic resistance, antibiotics susceptibility.

INTRODUCTION

The genus *Staphylococcus* belongs to the family Microcococeae. It is either a pathogen or can exist as normal flora of humans and other mammals (Prescott et al., 2005). Traditionally, they are divided into two groups based on the presence or absence of coagulase. The coagulase negative strains are mostly common commensals on skin, though some species can cause infection. *Staphylococcus aureus* expresses a variety of extra cellular proteins and polysaccharides, some of which are correlated with virulence (Brook et al., 1994). Using molecular DNA – ribosomal analysis of 16rRNA, it has been demonstrated that the initial genus, *Staphylococcus* now has about thirty species (Monica, 2002). *S. aureus* has been observed to contain many strains based on Polymerase Chain Reaction (PCR) analysis. The use of broth enrichment and real–time PCR is now employed to quantify *nuc gene* for MSRA (Methicillin Resistance *Staphylococcus aureus*) as a screening approach (Nilsson et al., 2005). *S. aureus* is the second largest (next to *Escherichia coli)* causative organism of nosocomial infections (Monica, 2002). Antibiotics resistance is on the increase among *S. aureus*. This has caused a great deal of concern.

In this research, *S. aureus* isolated from the septic caesaerean wound of a thirty-six years old woman were biochemically characterized. Antibiotics susceptibility patterns were determined.

*Corresponding author. E-mail: adejuwon_ao@yahoo.com, adejuwon_ao@walla.com. Tel: +2348069781680.

Table 1. Cultural characteristics of *Staphylococcus aureus* isolated from septic caesaerean wound on mannitol salt agar and nutrient agar.

Specimen code	Features on mannitol salt agar	Features on nutrient agar
O	Golden yellow colonies, profuse growth with circular smooth surface and regular edges.	Slightly yellow colonies, entire edge, smooth surface with botryous consistency and opaque.
D	Golden yellow colonies, circular convex, smooth and regular edges.	Slightly yellow in colour, flat moist and opaque.
K	Golden yellow, large convex and circular colonies.	Slightly yellow, flat, large circular and opaque.

Table 2. Cultural characteristics of *Staphylococcus aureus* isolated from septic caesaerean wound on blood agar and nutrient broth.

Specimen code	Features on blood agar	Features on nutrient broth
O	The growth was abundant round, small convex in elevation, shining with clear zones of haemolysis.	It became turbid after 18 h of incubation. It turned clear after 48 h with ring pellicles deposits at the bottom of the test tubes.
D	Colonies appeared golden yellow, circular, convex, smooth surfaces and zones of haemolysis were formed.	Highly turbid after 18 h of incubation at 37°C. Deposit pellicle after 48 h which become clear in the test tube.
K	Abundant growth, small convex, shining and zones of haemolysis were formed	Less turbid after 18 h of incubation at 37°C. Ringed pellicle (small amount) was formed after 48 h.

MATERIALS AND METHODS

Specimen collection

The collection of specimen for this research was done under aseptic conditions. The organisms used were isolated from swab sample collected from a thirty-six year old woman presented with septic caesaerean wound. This was done upon the consent of the patient. The patient was on admission at the Obafemi Awolowo Teaching Hospital Complex, Ile-Ife, Nigeria.

Isolation

The swabs were streaked on mannitol salt agar plates and incubated at 37°C for 24 h. The colonies which fermented mannitol appeared golden yellow in colour. They were subcultured into sterile peptone water (tubes) and nutrient agar (plates).

Media

All media used were from Lab M International diagnostic Group, Plc. They were *Staphylococcus* medium mannitol salt agar, nutrient agar, and blood agar. They were prepared according to manufacturer's instructions.

Biochemical tests and staining techniques

Biochemical tests carried out were grain stain, coagulase test, catalase test, Koser's citrate utilization test, methyl red Voges–Proskaeur (MRVP) test, indole production test and sugar fermentation tests. Other biochemical tests carried out were blood heamolysis test, gelatin liquefaction and Gram stain.

Antibiotics sensitivity test

Antibiotics sensitivity tests were carried out on the *Staphylococcus* positive strains. Diagnostic sensitivity test (DST) agar (Oxoid) and antibiotics multodisks Gram positive discs (Oxoid) were used.

RESULTS

Growth on mannitol salt agar

After incubation at 37°C for 24 h, the colonies appeared golden-yellow along the lines of streak on the mannitol salt agar. The colonies were large, convex, elevated, shinning, opaque and circular in appearance. Features on mannitol salt agar are given in Table 1.

Growth on nutrient agar

The colonies were large, circular and slightly yellow in colour after 24 h of incubation at 37°C (Table 1).

Growth on blood agar (haemolysis test)

After 24 h of incubation at 37°C, the zone of inhibition appeared as clear zones around each colony; the growth were abundant, round with small convex elevation (Table 2).

Table 3. Characterization of *Staphylococcus aureus* isolated from septic caesaerean wound.

Specimen code	MR	VP	Gram stain	Coagulase test	Catalase test	Citrate test	Gelatin hydrolysis	Blood haemolysis	Indole test
O	-	+	+	+	+	+	+	-	-
D	-	+	+	+	+	+	+	-	-
K	-	+	+	+	+	+	+	-	-

+: Positive reaction; -: Negative reaction.

Table 4. Fermentation by sugars *Staphylococcus aureus* isolated from septic caesaerean wound

Specimen code	Glucose	Sucrose	Galactose	Lactose	Mannitol
O	A	A	A	AG	A
D	AG	A	A	A	A
K	A	A	A	A	A

A= Acid production in positive result; AG= Acid and gas production in positive result.

Growth in nutrient broth

After the first 18 h of incubation at 37°C, growth was turbid, and at 44 h, changes to clear culture with the formation of ringed pellicles (Table 2).

Gram stain

The organisms appeared purple, coccoid in shape and were in clusters. They were Gram positive (Table 3).

Coagulase test

Slide method

There was clumping of the blood cells. They did not re-emulsify there after (Table 3).

Tube method

There was clotting of human blood plasma in the test tube after 3 h. The controls gave no change when observed (Table 3).

Citrate utilization

After 5 days of incubation at 37°C, with daily observation, the microorganisms changed the original green colour to blue from (Table 3).

Indole test

The test medium was incubated for 7 days at 37°C and observed. There were no changes in the original colour. The tests were negative for indole production (Table 3).

Catalase test

Slide method

The test portion of the slide showed the release of gas when 3% hydrogen peroxide was added in two drops. The results were positive (Table 3).

Tube method

The release of gas was vigorous on addition of four drops of the cultures into 3% hydrogen peroxide in test tubes. The results were positive for all the isolates (Table 3).

Methyl red and Voges-Proskaur test

After incubation at 37°C for 7 days, all the isolates in the culture gave negative result on both the methyl red test on addition of methyl red indicator and the Voges-Proskauer test on addition of Baritt's reagent (Table 3).

Gelatin liquefaction

After incubation at 25°C for 7 days, the protease gelatinase hydrolyzed the gelatin, which did not re-solidify when refrigerated. This gave positive result (Table 3).

Sugar fermentation

The experiments were incubated at 37°C for 5 days. There were changes in the colour of the tests. Acid and gas were produced (Table 4).

Table 5. Antibiotics sensitivity patterns of *Staphylococcus aureus* isolated from septic caesaerean wound.

Antibiotics	Conc (µg)	Zones of inhibition (mm)		
		O	D	K
Cephalexin	20	2.0	-	4.0
Drovid	10	7.0	4.0	5.0
Norfloxacin	10	6.0	1.0	14.0
Clindamycin	10	-	2.0	13.0
Septrin	25	3.0	1.0	1.0
Amoxillicin	20	-	-	-
Ampicloxacillin	20	1.0	1.0	-
Erythromycin	10	-	1.0	-
Gentamycin	10	-	-	1.0
Ciprofloxacin	5	1.0	10.0	16.0

Antibiotics sensitivity test

After 24 h incubation of the inoculated diagnostic sensitivity testing agar plates at 35°C, there were zones of inhibition around few antibiotics while majority did not have zones of inhibition. The zones were measured in millimeters and compared against standard to determine resistance and susceptibility. The antibiotic sensitivity is indicated in Table 5.

DISCUSSION

In this research, strains of *S. aureus* were isolated from a septic caesaerean wound of a 36 years old woman. *S. aureus* is one of the major microorganisms that cause post-operative wound infections (Apisarnthanarak et al., 2003), post war wound infection arising from injuries (Kalenic et al., 1991). Bowler (2002) reported the organism as a major factor affecting wound healing.

The strains of *S. aureus* obtained in this study were coagulase positive. However, this has been shown not to have direct virulence evidence in the host (Tenover et al., 1994). It is now known that coagulase is not an enzyme but an extracellular substance (Monica, 2002). The strains also haemolysed blood, producing complete clear zones which appeared colourless around each colony of *S. aureus*. This had been attributed to the presence of eight haemolytic factors (majorly in humans) (Bhakdi et al., 1991; Tenover et al., 1994). *S. aureus* had been characterized as a major human pathogen causing wide variety of diseases ranging from skin infection to life threatening septicemia, meningitis and toxic shock syndrome (Lowy, 1998). They also possess abilities to colonize and exploit the host functions and to cope with an often hostile host environment, thereby making them highly successful and opportunistic pathogens that are difficult to eradicate (Uziel et al., 2004).

The results of this study showed that there was susceptibility to cephalexin (a first generation cephalosporin), clindamycin (lincosamides), septrin (sulphonamides and trimethoprim) norfloxacin and ciprofloxacin (fluoroquinolones) and absolute resistance to gentamycin, erythromycin, ampicloxacillin and amoxicillin. It was only to drovoid that there was absolute susceptibility. Zhang (1992) reported that the size of zones of inhibition does not necessarily indicate relative sensitivity to antibiotics, since the zones are a function of inoculum size, solubility of the agents and the composition of the agar medium. However, many researchers have reported correlation between sizes of zones of inhibition and susceptibility (Geert, 2002; Eleonor, 2006). The inhibition on the basis of disk diffusion and the use of the Oxoid Aura Image Antibiotic sensitivity test system have been suggested (Andrews et al., 2000). This is because metre rule is tedious and error-prone.

According to Foster and McDevitt (2004), the development of resistance by *S. aureus* to antibiotics could have possibly arose by:

1. Acquisition of extrachromosomal plasmids or additional genetic information in the chromosome via transport or other types of DNA insertion, and
2. By mutation in chromosomal genes. This has been attributed to the prophylactic antibiotic treatment prior to surgical operation or cross contamination by resistant strains from the hospital environment.

The four major mechanisms of resistance as outlined by Prescott et al. (2005) are as follows:

1. Enzymatic inactivation of drug;
2. Alteration of the drug targets to prevent binding;
3. Accelerated drug efflux to prevent toxic concentrations accumulating in the cell and
4. A by-pass mechanism where by an alternative drug resistant version of the target is expressed.

As a result of the aforementioned factors, resistance to

gentamycin (amino glycoside) might arise due to permeability mutants and not as a result of the modifying enzymes transferase or the inactivating enzymes of the aminoglycosides by adenylation, acetylation or phosphorylation (Bertram, 2004; Edson and Terrell, 1987). If used on patient, it could lead to irreversible toxicity, which could lead to vestibular dysfunction and loss of hearing. It could also result in nephrotoxity that is reversible (Betram, 2004; Smith, 1980). Resistance to erythromycin is plasmid-encoded and `can arise by: (a) Reduced permeability of the membrane or efflux; (b) The production of esterase that hydrolyzed the macrolides and (c) The modification of the ribosomal binding site by chromosomal mutation or by a macrolide-inducible or constitute methylase (Spratt, 1994; Nikaido, 1994). Therefore prompt attention must be taken to remediate this resistance trend because the rapidity with which *S. aureus* can progress both in previously health individuals (if spread to them during health care delivery) and among patients is high (Robert, 2006).

It has been reported by Jack (1998) that nonviable *S. aureus* and its peptidoglycan can stimulate macrophage recruitment, angiogenesis, fibroplastia and collagen accumulation in wounded individuals. This could aid acute inflammatory response to wound healing and repair.

The development of faster diagnostic method of isolation culturing and testing antibiotics sensitivities and the use of broth enrichment media with real-time PCR to exclude the presence of methicillin-resistant *S. aureus* have been recommended (Nilsson et al., 2005). The development of new antibiotics can be of great help. For example, exploiting the structural differences between the bacteria and mammalian thioredoxin reductase can serve as a target for the development of new antimicrobials (Uziel et al., 2003).

CONCLUSION AND RECOMMENDATION

Conclusively, from this study, it is evident that the *S. aureus* strains found in the septic wound exhibited multiple resistances to antibiotics, exploting the immunocompromised state of the patients. This may lead to multiple infections or diseases such as *S. aureus* associated-pneumonia, septicemia, osteomyelitis, ritta disease, scalded skin-syndrome, thrombophlebitis and empyemia (Robert, 2006). These would increase hospital stay and additional financial cost of treatment especially because of the multidrug-resistance.

The spread of pathogenic *S. aureus* in the hospital environment ranging from contaminated surface, general utility devices and health workers to the patients or vice-verse can be appropriately reduced or eradicated by regular decontamination of implicated devices, surfaces and good personal hygiene of the health care workers and patients. Indiscriminate use of antibiotics should also be discouraged. The use of sterile wound dressing materials should be enhanced during wound dressing. Implementation of standard precautions and contact precautions will also greatly reduce cross contaminations through spread from both patients and healthy workers. The hospital management can set up epidemiological surveillance and regular intensive microbiological survey departments in the hospitals. The use of new antibiotics and quicker methods or approaches of screening patients and health worker carriers of resistant microorganisms will be of immense help.

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