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Short Communication

Antibiotics resistance and susceptibility pattern of a strain of *Staphylococcus aureus* associated with acne

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Staphylococcus aureus **was isolated from an individual with acne. The isolate was Gram positive, spherical and in clusters, golden yellow on mannitol salt agar and non spore forming. Biochemical tests showed that it was unable to hydrolyze gelatin but able to ferment glucose, galactose and mannitol. It produced catalase and coagulase enzymes. It was able to utilize citrate as sole carbon source but was indole negative. It was Methyl Red positive and Voges-Proskauer negative. It was sensitive to gentamicin, tetracycline, amoxicillin, augmentin, chloramphenicol and sulphamethoxazole but resistant to ampicillin, erythromycin, cloxacillin, cotrimoxazole, streptomycin and penicillin.**

Key words: Antibiotics, resistance, susceptibility, acne, *Staphylococcus aureus*.

INTRODUCTION

Acne is a disorder of the sebaceous glands of the skin in which sebum secretions is excessive. The glands become plugged and inflamed in the process (Vogt et al., 1992). Acne also referred to as pimple can also be defined as a clinical condition brought about by the blockage of the pilosebaceous follicles of the face and the upper trunk (Olumide, 1993). In dermatological terms, acne or pimple can be defined as papule, pustule, nodule or cyst (Leyden, 1997) . A papule is a raised lesion, one centimeter in diameter while a pustule is a similar lesion containing pus. Nodules are lesions that are one centimeter or larger (often extending deeper or higher and firm in touch). Cysts are nodules filled with fluid, often pus (San-Jay, 2004).While acne is frequently a transient problem observed mainly in the adolescent years, some manifestations of the disease can last forever (Papadopoulous and Walker, 2004).

Staphylococci are Gram positive, spherical bacteria (0.5 - 1.5 m in diameter) which occur singly, in pairs or tetrad. They are short chained with three to four cells forming irregular clusters (Brock et al., 2001). Members of this genus are facultative anaerobes, chemoorgano- trophic with both respiratory and fermentative metabolism (Brock et al., 2001). They are usually catalase positive and associated with skin and mucous membrane of vertebrates (Prescott et al. , 2005). They can be found in the bacteriological cultures of the nose and skin of normal humans (Brock et al., 1994). Some are members of the normal flora of the skin and mucous membranes of humans; others cause suppuration, abscess, a variety of pyogenic infections and fatal septicemia (Brock et al., 2001). In humans, two species are important, *Staphylococcus epidermidis*: a non pigmented, non-pathogenic form, usually found in the skin and mucous membrane and *Staphylococcus aureus*: a yellow pigmen-ted form associated with pathological conditions such as boils, pimples and impetigo (Brock and Madigan, 1991).

This study was designed to isolate *Staphylococcus aureus* from existing acne in an individual. Antibiotics resistance and susceptibility pattern of this isolate were determined. Possible control measures against the development of antibiotics resistance strains is discussed.

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Table 1. Morphological and cultural characteristics of *Staphylococcus aureus* associated with acne.

Feature	Appearance
Shape	Spherical (coccoid) and in clusters.
Growth on nutrient agar	Moist, white, opaque colonies with entire edges and smooth butyrous consistency.
Growth on mannitol salt agar	Profuse growth of yellow flat colonies with regular edge and smooth surface.
Gram	+
Spore	-

Key: + = positive; - = negative.

MATERIALS AND METHODS

Media preparation

Some of the media used were of the LAB M, International Diagnostic Group Plc. They were Mannitol salt agar, Nutrient agar and Koser's citrate media. Diagnostic Sensitivity Test (DST) agar (Oxoid) was also used. All media were prepared according to the manufacturers' specifications and sterilized at 121°C for 15 min in an autoclave.

Collection of sample

Collection of sample was done under aseptic conditions. The surface area of pimple from a 22 year old male individual was disinfected by cleaning with cotton wool soaked in 75% alcohol. The pimple was pressed and the pus taken with a sterile swab.

Isolation of *Staphylococcus aureus*

Pus taken with sterile swab was inoculated onto plates of Mannitol salt agar medium. The plates were incubated at 37°C for 24 h. After incubation, the colonies which had fermented mannitol and which appeared golden yellow were sub cultured onto nutrient agar slants for further characterization.

Stains

Gram stain and spore stain were carried out on 24 h old cultures of the isolate. Malachite green and safranin were prepared for the spore stain.

Biochemical tests

Biochemical tests carried out were catalase test, citrate utilization test, indole test, Methyl Red and Voges-Proskauer test, gelatin hyrolysis and fermentation of sugars.

Antibiotics sensitivity test

Diagnostic Sensitivity Test agar (Oxoid) was prepared in Petri dishes according to manufacturer's specifications. Antibiotics multodisks (Oxoid) used were of two types: Multodisk code GBMTS POS and multodisk code1789E.

A loopful of 24 h old culture was placed at one end of the agar surface in Petri dishes. A sterile swab was used to spread the organism uniformly on the surface of medium. Using sterile forceps, a multodisk was placed in the centre of the medium. The Petri dishes were incubated at 37°C for 24 h in an inverted position. Diameter of zone of inhibition around each antibiotics disc was recorded in millimetres.

Clear zones of inhibition more than 2 ml on either side of the antibiotics disc was taken as sensitive while zones less than 2 ml were considered to be resistant.

RESULTS

Pus collected aseptically from individual and streaked on Mannitol salt agar showed colonies which fermented mannitol and appeared golden yellow. Cultural characteristics indicate the organism to be spherical, in clusters and gram positive. The organism did not produce spores (Table 1). On nutrient agar, growth was opaque, smooth, flat, moist and white in colour (Table 1).

Catalase, indole and citrate utilization tests

The organism was catalase positive and after incubation for 5 days at 37°C, there was no colour change in the Kovac's indole reagent, indicating that the isolate was indole negative. On the Koser's citrate medium, there was a colour change from green to blue. This showed that the isolate utilized citrate as the sole carbon source (Table 2).

Methyl red, Voges-Proskauer and gelatin hydrolysis tests

A colour change in the medium on the addition of Methyl Red reagent indicated that the organism was Methyl red positive. There was no colour change in the medium for Voges-Proskauer test on the addition of 6% -naphthol followed by Potassium hydroxide, indicating that the organism was negative for Voges-Proskauer test (Table 2).

The isolate was unable to hydrolyze gelatin as no clear zone was observed after the addition of mercuric chloride solution (Table 2).

Table 2. Biochemical characteristics of *Staphylococcus aureus* associated with acne.

Test	Observation
Catalase	+
Indole	-
Citrate	+
Methyl red	+
Voges-proskauer	-
Gelatin hydrolysis	-

Key: + = positive: - = negative

Table 3. Sugar fermentation test on *Staphylococcus aureus* associated with acne.

Fermentable sugar	Observation
Glucose	A
Galactose	A
Lactose	A
Maltose	A
Sucrose	A
Mannitol	A
Starch	NA

Key: A = Acid production; NA = No acid production.

Fermentation of sugars

Production of acid during fermentation was indicated by the change in the colour of the indicator (Phenol red) to yellow. Displacement of air in the inverted Durham tubes showed production of gas. Inability to produce gas during fermentation of sugars was indicated by the absence of gas in the inverted Durham tubes. The isolate fermented glucose, galactose, lactose, maltose, sucrose and mannitol with acid formation without gas production. However, on starch, as the sole carbon source, neither acid nor gas was produced (Table 3).

Antibiotics sensitivity

The isolate was sensitive to gentamicin, tetracycline, amoxicillin, augmentin, chloramphenicol, sulfamethoxazole. However, it was resistant to ampicillin, erythromycin, penicillin, streptomycin, cloxacillin and cotrimoxazole (Table 4).

DISCUSSION

The results of this investigation showed the presence of *Staphylococcus aureus* in pus from acne. *Staphylococcus aureus* is the common organism associated with pus formation in acne (Brock et al., 1994)

Pathogenicity of *Staphylococcus aureus* in pimple has been attributed to virulence factors possessed by the organism (Beamer, 2002). These virulence factors include coagulase, leukocidins, Protein A (an Fc binding protein), hyaluronidase, hemolysin, lipase and a variety of toxins such as enterotoxin and dermonecrotin toxin (Office of Medical Informatics, 1999).

The organism fermented mannitol, appearing as small, yellow, smooth, shining colonies. The ability of *Staphylococcus aureus* to grow on mannitol salt agar could be attributed to its ability to grow on relatively high concentrations of sodium chloride, as contained in the medium (Nester et al., 1998; Adejuwon and Ajisebutu, 2004). Sweat with some quantity of salt on the face and upper trunk of individuals constitutes a good medium for the growth of *Staphylococcus aureus* (Lowy, 1998).

Antibiotics sensitivity tests carried out in this research indicate that the organism was resistant to ampicillin, penicillin, erythromycin, streptomycin, cloxacillin, and cotrimoxazole. The strain was however sensitive to gentamicin, tetracycline, amoxicillin, augmentin, chloramphenicol and sulfamethoxazole.

Resistance to antibiotics has become a serious problem in recent years, particularly the rise in epidemic strains of methicillin resistant *Staphylococcus aureus* (MRSA). Glycyclines and some synthetic vancomycin derivatives with modified disaccharides have been found to be highly

Table 4. Antibiotics sensitivity pattern of *Staphylococcus aureus* associated with acne.

Multodisk code	Antibiotics code	Concentration (g)	Zone of inhibition (mm)
	PN	2	0
1789E (Oxoid)	C	10	0
	OB	5	0
	E	10	0
	P	1.5	0
	S	10	0
	TET	10	0
	SXT	25	15
	GEN	10	24
	TET	10	13
	AMX	25	16
GBMTS POS (Oxoid)	AUG	30	21
	CHL	30	11
	COT	25	0
	CXC	5	0
	ERY	5	0

Key: PN, Ampicillin; C, Chloramphenicol; OB, Cloxacillin; P, Penicillin; G, Streptomycin; TET, Tetracycline; SXT, Sulfamethoxazole; GEN, Gentamicin; AMX, Amoxicillin; AUG, Augmentin; CHL, Chloramphenicol; COT, Cotrimoxazole; CXC, Cloxacillin; E, Erythromycin; ERY, Erythromycin.

active against methicillin resistant strains of *Staphylococcus aureus* (Zhanel et al., 2004; Akiyama et al., 1998).

Acne therapy varies according to the severity of the disease. Topical medications are generally adequate in cleaning comedonal acne, while inflammatory acne usually requires addition of oral medication (Day, 2004). Systemic antibiotics used most frequently have been found to be highly effective (Watcher, 2004).

The results of this study have shown a multiple antibiotics resistance feature of a strain of *Staphylococcus aureus* associated with acne. With these findings, we suggest that indiscriminate use of antibiotics, which predisposes individuals to the development of antibiotics resistant pathogenic strains, should be avoided.

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