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Research Article

Studies on *Staphylococcus aureus* Isolated from Pimples

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Abstract

Background and Objective: Pimples (acne) are small skin lesions or inflammations of the skin. The most common factor causing acne is the hormonal changes that occur during adolescent and teenage years. Antibiotics are becoming less effective in the treatment of pimples due to increasing concerns of antibiotic resistance. This study was therefore carried out to characterize the isolates from the pimples of Covenant University Students and to determine their antibiotics sensitivity pattern. **Materials and Methods:** A total of 20 swab samples were obtained from male and female students with obvious signs of pimples in Covenant University, Ota, Ogun State, Nigeria. The samples obtained were cultured on Mannitol Salt Agar and incubated at 37°C. Pure isolates obtained were subjected to Gram staining and other biochemical tests for identification. The isolates were further subjected to antibiotics sensitivity tests using antibiotic discs. **Results:** Macroscopic examination indicated that the organisms were convex, smooth and shiny. Microscopic examination revealed that the isolates were positive after employing the Gram Staining technique and they appeared as grape-like clusters. Biochemical tests revealed that the isolates were Coagulase positive, Catalase positive, Urease positive, Citrate positive, Methyl-Red positive, Voges-Proskauer negative and negative upon starch hydrolysis. The sugar fermentation tests revealed that the isolates fermented Glucose, Maltose, Galactose, Sucrose and Lactose, respectively. The antibiotic susceptibility test showed that isolates were resistant to Cotrimazole, Cloxacillin, Erythromycin, Gentamycin, Augmentin, Streptomycin, Tetracycline and Chloramphenicol. **Conclusion:** The results therefore indicated that the isolates were *Staphylococcus aureus* and other staphylococci species. Indiscriminate use of antibiotics should be avoided to prevent the development of resistant strains of the Staphylococci genera and other pathogenic organisms.

Key words: *Staphylococcus aureus*, antibiotic susceptibility, pimples, biochemical tests, acne, morphological characterization

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pimple is the common name for a disorder that causes outbreaks of skin lesions known as acne¹. Acne is a disorder of the sebaceous glands of the skin in which sebum secretions are excessive. The glands become plugged and inflamed in the process^{2,3}. Acne is also known as *Acne vulgaris*. It is one of the most common skin diseases, affecting approximately 85% of people between the ages of 12-25^{4,5}. It is estimated that 45 million people in the United States have acne, with a prevalence of approximately 85% in the population between 15 and 24 years of age^{5,6}. Skin and soft tissues infections are usually caused by *Staphylococcus aureus* and *Streptococcus pyogenes*^{7,8}. Large portions of these infections are caused by normal skin flora and the nature and severity of the infection is dependent upon the site of inoculation and type of microorganism⁹. If the infection is left unattended to the organism, may spread to the surrounding tissues and the tiny lesion becomes a much larger, a swollen, cutaneous, inflamed, localized lesion walled by deposition of fibrin by the tissue and the walling off is to prevent the Staphylococcal infection from going further¹⁰. This study was therefore carried out to randomly sample the pimple present on faces of students of Covenant University for the presence of *Staphylococcus aureus*, to isolate and characterize the strains of *Staphylococcus aureus* obtained from the student's pimple and determine the antibiotic susceptibility profile for the strains of *Staphylococcus aureus*. This will aid in the effective treatment of *staphylococcal* pimples infections.

MATERIALS AND METHODS

Sample collection: Samples were collected from male and female students of Covenant University, Ota, Nigeria. The collection was carried out under aseptic condition using sterile swab sticks. The pus of the pimple was pressed out and taken with the sterile swab sticks. The swabs containing the inoculum were streaked on sterile Mannitol Salt Agar plates. The inoculated plates were then incubated at 37°C for 24 h. After incubation, the colonies which appeared golden yellow were isolated and sub-cultured on nutrient agar slants.

Grams staining test: A heat fixed smear from a 24 h old culture was prepared on a clean glass slide. The smear was stained with Crystal Violet solution for 1 min after which the solution was rinsed off with water. The slide was flooded with Lugol's Iodine for 1 min. The iodine was rinsed off with water and then the slide was decolorized using 70% Ethanol for

30 sec. The slide was rinsed gently with water and counter stained with safranin solution for 1 min. The slide was washed with water and then air dried. The slide was observed under the microscope using the oil immersion objective lens.

Biochemical tests: The isolates were subjected to the following biochemical tests, catalase test, coagulase test, citrate utilization test, urease test, methyl red test, Voges-proskauer test, starch hydrolysis and sugar fermentation tests.

Antibiotic sensitivity testing: The disc diffusion method was used to determine the susceptibility and resistance of the organisms to the antimicrobial drugs. The inoculum was streaked on Mueller Hinton agar plates and a Gram positive antibiotic multi disk (commercial drug disc containing antibiotics with different concentrations) was aseptically removed and placed at the centre of the plate. The plates were incubated at 37°C for 24 h and the diameter of zones of inhibition formed around each antibiotic disc was measured.

RESULTS AND DISCUSSION

Morphological and cultural characteristics: This study revealed that the isolates from the pimples swabs of twenty male and female students between the ages of 16-24 years streaked on Mannitol salt agar showed colonies which fermented Mannitol and appeared golden yellow (Table 1). Cultural and morphological characteristics indicated that the colonies are spherical in shape, appeared in clusters, non-spore forming and were Gram positive (Tables 2, 3). Biochemical tests carried out revealed that the isolates are

Table 1: Incidence of Pimples in relation to age and sex of the students

Age range	No of individual with pimple	(%) of occurrence	Sex	Total positive for <i>S. aureus</i>	Positive (%) for <i>S. aureus</i>
16-18	3	60	F	5	25
	2	40	M		
19-21	4	40	F	10	50
	6	60	M		
22-24	3	40	F	5	25
	2	60	M		

F: Female, M: Male

Table 2: Morphology and Cultural Characteristics of *Staphylococcus aureus* associated with Students pimple

Features	Appearance
Colour	Yellow
Elevation	Convex
Margin	Entire
Gram's stain	Positive, purple colour, coccoid, in pairs and grape-like clusters

Table 3: Gram's staining reaction and Biochemical tests of the strains of *S. aureus* isolated from the pimple of students

Code	Age	Gram stain	Coagulase test	Catalase test	Urease test	Citrate test	Methyl red	Starch	Voges-Proskauer
F1	16	+	+	+	+	+	+	-	-
F2	21	+	+	+	+	+	+	-	-
F3	21	+	+	+	-	+	+	-	-
F4	17	+	+	+	+	+	+	-	-
F5	23	+	+	+	+	+	+	-	-
F6	23	+	+	+	+	+	+	-	-
F7	20	+	-	+	+	+	+	-	-
F8	22	+	+	+	+	+	+	+	-
F9	19	+	+	+	+	+	+	-	-
F10	18	+	+	+	+	+	+	-	-
M1	17	+	+	+	+	+	+	-	-
M2	19	+	-	+	+	+	+	-	-
M3	19	+	+	+	+	+	+	-	-
M4	18	+	+	+	+	+	+	-	-
M5	21	+	-	+	+	+	+	-	-
M6	22	+	+	+	-	+	+	-	-
M7	20	+	+	+	+	+	+	-	-
M8	21	+	+	+	+	+	+	-	-
M9	23	+	+	+	+	+	+	-	-
M10	21	+	+	+	+	+	+	-	-

KEY, F: Female M-Male, +: Positive, -: Negative

Table 4: Sugar Fermentation Properties of the Strains of *S. aureus* Isolated from the Students Pimple

Code	F1	M1	F2	M2	F3	M3	F4	M4	F5	M5	F6	M6	F7	M7	F8	M8	F9	M9	F10	M10
Glucose	A	A	A	A	A	AG	A	A	A	AG	AG	A	A	A	A	A	A	A	A	A
Maltose	A	A	AG	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Galactose	AG	A	A	A	A	A	A	A	A	A	A	A	A	AG	A	A	A	A	A	A
Sucrose	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Lactose	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	AG	A	A	A

A: Acid production, G: Gas production, AG: Acid and gas production

Table 5: Antibiotic Sensitivity Pattern of *S. aureus* Stains isolated from the pimples of students

Antibiotics (mm)	Specimen code																			
	1		2		3		4		5		6		7		8		9		10	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
COT	0	8	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	2	0
CXC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ERY	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	2	0	0	0
GEN	3	2	8	1	0	1	0	1	7	0	0	2	1	2	3	2	6	5	2	2
AUG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STR	0	0	0	0	6	4	0	4	0	0	0	0	5	0	5	1	0	0	0	0
TET	1	2	6	0	3	0	0	0	3	0	0	0	0	2	0	1	1	0	0	0
CHL	6	0	7	0	8	0	5	0	6	1	0	0	8	5	6	5	8	6	6	0

KEY, COT: Cotrimazole CXC-Cloxacillin, STR: Streptomycin, TET: Tetracycline, GEN: Gentamycin, CHL: Chloramphenicol, ERY: Erythromycin, AUG: Augmentin

Coagulase positive, Catalase positive, Urease positive, Citrate positive, Methyl-Red positive, Voges-Proskauer negative and they were negative upon Starch hydrolysis (Table 3). The sugar fermentation tests revealed that the isolates fermented Glucose, Maltose, Galactose, Sucrose and Lactose as shown (Table 4). The antibiotic sensitivity test showed that the isolates were resistant to cotrimazole, cloxacillin, erythromycin, gentamycin, augmentin, streptomycin, tetracycline and chloramphenicol (Table 5, 6). The results revealed that all the isolates are *S. aureus* strains and had

least resistance to gentamycin and greater resistance to cloxacillin and augmentin.

The result of this investigation revealed that the isolates were *Staphylococcus aureus*. *Staphylococcus aureus* forms a normal part of the human skin¹¹. *S. aureus* is found on the face and hands, particularly in individuals who are nasal carriers¹². Although *Staphylococcus aureus* is a normal inhabitant of the skin, mucous membranes, respiratory and gastrointestinal tracts, it can invade any organ or system to produce infection ranging from localized to invasive diseases¹³. Localized

Table 6: Antibiotic Resistance Pattern of *S. aureus* strains isolated from pimples of Covenant University Students

Antibiotics	Number of isolates resistant	Resistance (%)
Clotrimazole	17	85
Cloxacillin	20	100
Erythromycin	18	90
Gentamycin	4	20
Augmentin	20	100
Streptomycin	14	70
Tetracycline	12	60
Chloramphenicol	7	35

diseases include furuncles, impetigo, boils and other wound infections are community acquired. Suppurative and or invasive infections include septicemia, osteomyelitis, arthritis, endocarditis and pneumonia which is hospital acquired⁴. The species of *Staphylococcus aureus* isolated from the pimple of students on which different tests were carried out revealed the organism as a yellow, smooth, shining colonies on Mannitol salt agar and also appeared coccoid in shape and gave cultural and morphological characteristics consistent with those of *Staphylococcus aureus*. *S. aureus* has been implicated as a major organism associated with pus from acne^{14,15}. The pathogenicity of *Staphylococcus aureus* in pimples has been attributed to the virulence factors possessed by the organism¹². Michels *et al.*¹⁶ and De Luca and Valacchi¹⁷ reported that there are some biological changes on the skin surface lipids which allow the inflammation of acne and that the bacterial colonization of sebaceous follicle results in the production of inflammatory agents. The antibiotic sensitivity tests carried out indicated that the organism was resistant to gentamycin and chloramphenicol, implying the possibility of high efficiency of these antibiotics in the treatment of pimples. The strains were however sensitive to clotrimazole, tetracycline, cloxacillin, erythromycin, augmentin and streptomycin. The results revealed multiple antibiotic resistance of *Staphylococcus aureus* associated with pimple. Adejuwon *et al.*¹² reported that the *Staphylococcus aureus* isolated from an individual was sensitive to gentamicin, tetracycline, amoxicillin, augmentin, chloramphenicol and sulphamethoxazole but resistant to ampicillin, erythromycin, cloxacillin, cotrimoxazole, streptomycin and penicillin. Ajayi *et al.*¹¹ also reported that the *staphylococcus* isolates isolated from hand swabs obtained from female and male students' palms at Covenant University, Ota were susceptible to ciprofloxacin, gentamycin, pefloxacin and amoxycillin but resistant to erythromycin, ceftriazone and cotrimoxazole antibiotics.

CONCLUSION AND FUTURE RECOMMENDATIONS

This study revealed that the *S. aureus* isolated from pimple swabs of students showed a high resistance to all the

antibiotics tested. Therefore, indiscriminate use of antibiotics should be avoided and where antibiotics are to be administered, the choice of antibiotics in any given case should be based on the results of antibiotic sensitivity tests. Practicing clean personal hygiene is encouraged as an effective method of preventing the transmission and occurrence of pimples and other staphylococcal infections. Washing of the face regularly with antiseptic soaps and the use of hand sanitizers is also encouraged. Wounds and cuts should be properly covered and sharing of personal items such as clothing and towels, should be avoided. Awareness should be highly recommended amongst students of higher institutions particularly among the age group tested.

SIGNIFICANCE STATEMENT

This research work revealed isolated microorganisms from male and female students with obvious signs of pimples and discovered that the isolates were *staphylococcus* species after characterization. The study therefore recommends that the indiscriminate use of antibiotics should be avoided to prevent the development of antibiotic resistant strains of staphylococci genera and other pathogenic organisms.

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