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IDENTIFICATION AND SUSCEPTIBILITY PATTERNS OF SOME SKIN BACTERIA ISOLATES USING VITEK-2 SYSTEM

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ABSTRACT

The use of the automated Vitek-2 system as a sensitive tool in the identification and antibiotic susceptibility of microorganisms cannot be overemphasized especially for its fast, efficient and reliable technology. This study evaluated the Vitek-2 system for identification and susceptibility pattern of some isolated bacteria from skin infections. Two hundred and seventy-two skin swab samples from pupils with skin infections in some selected primary schools across the three geopolitical zones of Ogun State were examined for this study. Isolation and comparison of bacteria identification and susceptibility pattern were achieved using standard microbiological techniques, agar disc diffusion method and Vitek-2 system, respectively. Out of the two hundred and seventy-two (272) skin swab samples collected, twenty-eight (28) were identified to specie-level by Vitek-2 system. *Staphylococcus aureus, Staphylococcus hominis, Staphylococcus cohnii, Staphylococcus saprophyticus, Staphylococcus epidermidis, Staphylococcus xylosis, Staphylococcus agalactiae and Staphylococcus lugdunensis* were the definitive species of *Staphylococcus identified. Staphylococcus aureus* 13 (46.43%) had the highest prevalence of identity, however, *Staphylococcus lugdunensis*, *Staphylococcus cohnii* and *Staphylococcus xylosis* had the least occurrence of 1(3.57%) each. The isolates were highly susceptible to ciprofloxacin 24(85.71%), followed by gentamycin 21(75%) and nitrofurantoin 20(71.43%). All the isolates were absolutely resistant to benzylpenicillin 28(100%). A significant increase in the zone of inhibition of ciprofloxacin when compared with cefuroxime (F-ratio=9.325, P-value <0.05) was observed using the agar disc diffusion method. The study concluded that the Vitek-2 system is a definitive and comprehensive technique for bacteria identification and antibiotic susceptibility, thus providing rapid and accurate results while saving time and work.

Keywords: Vitek-2 system, bacteria; isolates; infections; skin.

INTRODUCTION

Identification of microorganisms has been an essential aspect of microbiology, and beyond the conventional methods, some automated systems have been developed for accurate and rapid effective treatment of infections. Rapid bacterial identification and antimicrobial susceptibility testing due to advances in technology have been recog-

nized to be of clinical and financial benefits. The use of automated systems for microbial identification has reduced the duration of analysis and the ability to simultaneously determine several microorganisms, while retaining accurate results as been advantageous (Buszewski *et al*., 2017).

The Vitek system was introduced around 1970 as an automated system for identification (ID) and antimicrobial susceptibility testing (AST) of bacteria isolates. This has however evolved today in to Vitek-2 system, which automatically performs all the required procedures for microbial identification and antimicrobial susceptibility testing after a primary inoculum has been prepared and standardized (Ligozzi *et al*., 2002). This system allows kinetic analysis by reading each test every 15 min. The optical system combines multichannel fluorimeter and photometer readings to record fluorescence, turbidity, and colorimetric signals (Ligozzi *et al*., 2002).

The microbial identification and antimicrobial susceptibility testing by Vitek-2 has been associated with reduced turn-around-time for sample analysis and detection of multi-drug resistant organisms, thus, it requires less manipulation time and results are available faster (Warenn, 2015). The ID and AST principle of the automated system is based on miniature biochemical and chromogenic tests and broth micro-dilution methods, respectively.

Staphylococcus aureus is an opportunistic pathogen and the most common cause of skin and soft tissue infections (Akinduti *et al*., 2022). According to Stanley (2013), the various infections caused by *Staphylococcus aureus* have been on the increase globally with serious implications for public health. In 2018, Ghalehnoo reported that *Staphylococcus aureus* is a common cause of skin and skin structure infections as well as osteoarticular infections in the human population. The severity of Staphylococci infection ranges from mild skin abscess and superficial tissue infections to life threatening diseases. Folliculitis, furuncle, impetigo and carbuncle are some of the common topical infections caused by *Staphylococcus aureus* (Ghalehnoo, 2018).

According to Akinduti *et al.* (2022), the global epidemiological reports have shown that skin and soft tissue infections are usually aggravated by *Staphylococcus aureus* bioflm, usually leading to extensive antibiotic resistance, thereby limiting available treatment options. Therefore, an increase in the skin infections caused by *staphylococcus aureus* has been of public health concern (Ghalehnoo, 2018) and the stigmatization among children is on rage.

The gram staining, catalase test, coagulase test, citrate test and growth on Mannitol Salt Agar (MSA) are the routine conventional methods of identifying *Staphylococcus spp.* However, these methods are time-consuming and do not give a definitive identification to the species and strain level. Due to lack of adequate facilities or financial implications in developing countries and poor resource settings, other members of the *Staphylococcus spp* may have been erroneously identified as causative agents for a variety of disease conditions (Stanley *et al*., 2013). This study aim at identifying some skin bacteria isolates to specie level and to determine the susceptibility patterns of the isolates using Vitek-2 system.

MATERIALS AND METHODS

Ethical approval

Ethical approvals were obtained from Health Research and Ethical Committee (HREC) of Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State (OOUTH/HREC/287/2019AP) and the State Universal Basic Education Board (SUBEB/PRS/815/Vol.2/476), Abeokuta, Ogun State, respectively.

Sample collection

A total of 272 skin swab samples were aseptically collected from primary school pupils with skin lesion at different parts of the body, across six (6) different government schools in Ogun state between January and June 2021. Skin swabs were collected by using sterile cotton swab sticks and the skin was decontaminated with 70% alcohol to remove surface bacterial contamination. The cotton swabs were moistened with sterile normal saline and the moistened cotton swabs were rubbed gently over the site of infection for about 15-30 secs and placed in tube containing nutrient broth for laboratory investigations (Myles *et al*., 2016).

Isolation and identification of bacteria

A selected agar medium, Mannitol Salt Agar (MSA) was prepared according to manufacturer instructions and allowed to solidify. 0.1ml of the collected skin swab sample broth was inoculated on the surface of the agar using spread plate method. This was incubated at 37^0C for about 24hrs. According to Adetayo *et al.* (2014), *Staphylococcus spp* produces a quite number of pigmentations ranging from white to gold yellow on MSA. The bacteria isolates were further identified morphologically and biochemically by gram staining, catalase, oxidase and coagulase tests respectively.

Gram stain test

A slightly modified procedure of Owolabi *et al.* (2020) was adopted for this study. A heat-fixed smear of the bacteria colony was covered with crystal violet, rinsed with water and stained with Gram's iodine. A rapid decolorization with alcohol for few seconds, rinsed with water and counterstained with safranin for 30seconds was performed. The smear was rinsed with water, blot dry and examined under oil immersion objective. The cell wall of gram-positive bacteria appeared blue or purple in colour (Smith *et al*., 2016).

Oxidase test

Oxidase test was carried out using a freshly prepared reagent solution. Kovacs Oxidase Reagent was dissolved in distilled water and few drops were released on filter paper. Colonies of bacteria were picked using an applicator stick and rubbed on the surface of the filter paper. A change in colour indicates positive reaction while no colour change indicates negative reaction (Azeez *et al*., 2020). Organisms that contain cytochrome-C produces oxidase enzyme which turns the reagent blue or purple (Dharmappa *et al*., 2022).

Catalase test

Few colonies of the bacteria emulsified on a clean glass slide is placed in a petri dish and few drops of hydrogen peroxide was added (Owolabi *et al*., 2020). The catalase test is used to detect the presence of catalase enzyme that decomposes hydrogen peroxide to form water and oxygen. An immediate formation of bubbles validates a positive test (Khatoon *et al*., 2022).

Coagulase test

Coagulase test was performed on a clean glass slide and colonies of the isolates were emulsified. A drop of plasma was added and

formation of clumps was observed as positive test result (Owolabi *et al.*, 2020). The presence of plasma will cause the bacterial cell to clump, thus, a bound coagulase is present on the bacterial cells (Katz, 2016).

Agar disc diffusion method

Antimicrobial susceptibility test was carried out using Kirby Bauer disc diffusion method. Mueller Hinton agar plates were prepared according to manufacturer's instructions and 0.5 McFarland standard broth suspensions of bacteria were inoculated. A multidisc (Rapid Labs, UK) containing various antibiotics were placed on the surface of the inoculated plates and incubated at 37^0C for about 24hours. The zones of inhibition were measured in millimeter (mm) using a graduated metre rule following the procedures of Azeez *et al.* (2020).

Identification and antibiotic susceptibility tests using Vitek-2 system

The identification and antibiotic susceptibility tests using the Vitek-2 (BioMérieux version 8.01) compact system were performed at the University College Hospital, Ibadan, Nigeria. The system uses a fluorogenic methodology for organism identification and a turbidimetric method for susceptibility testing, using a 64 well card that is barcoded with information on card type, expiration date, lot number and unique identification number (Benkova *et al.*, 2020).

Vitek-2 Compact system was used according to the manufacturer's instructions. The suspension preparation for the identification cards was achieved by transferring 3ml of Saline into a tube. A pure colony was selected and suspended in the saline tube and the content was well mixed. The optical density of the mixture was checked with the DensiCHEK TM while the identification (ID) cards and tubes were placed into the cassette. Moreover, the antibiotic susceptibility test (AST) suspension was prepared by transferring 3 ml of Saline into a tube and transfer 280 µl (gram positive or yeast) or 145 µl (gram negative) of the ID suspension into the Saline tube, after which the antibiotic susceptibility test cards and tubes were placed in to the cassette. The cassettes were loaded into the instrument, the Fill door was closed and the device automatically performs the ID and AST analysis (Benkova *et al.*, 2020).

Data analysis

Data were analyzed using analysis of variance and means were separated by Duncan's multiple range test ($p < 0.05$).

RESULTS

About 86% of the bacteria were positive to gram stain test, 89.29% were coagulase positive, 85.71% were negative to oxidase test while all the organisms (100%) were positive to catalase test (Table 1). The bacteria were mostly susceptible to ciprofloxacin (53.57%), closely followed by Ofloxacin (46.43%), however, the bacteria displayed more resistance (82.14%) to cefurozime (Table 2). A significant increase was observed in the mean zone of inhibition of ciprofloxacin (16.210±2.215) when compared to other antibacterial agents (F-ratio=9.325, P-value <0.05) – Table 2.

Staphylococcus saprophyticus, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus lugdunensis, Streptococcus agalactiae, Staphylococcus cohnii and *Staphylococcus xylosis* were identified to specie level by Vitek-2 system (Fig. 1). Out of the species identified, *Staphylococcus aureus* 13(46.43%) was predominant, closely followed by *Staphylococcus saprophyticus* 5(17.86%), *Streptococcus agalactiae* 3

(10.71%), *Staphylococcus hominis* 2 (7.14%) and *Staphylococcus epidermidis* 2 (7.14%). However, the least identified specie were *Staphylococcus lugdunensis* 1(3.57%), *Staphylococcus cohnii* 1(3.57%), and *Staphylococcus xylosis* 1 (3.57%), respectively (Fig. 1).

Most of the identified bacteria isolates were susceptible to the antibacterial agents but few exhibited intermediate values to vancomycin 1(3.57%), tetracycline 3(10.71%) and nitrofurantoin 4(14.29%) –Table 3. Ciprofloxacin 24(85.71%) had the highest percentage of susceptibility followed by gentamycin 21(75%) and nitrofurantoin 20(71.43%). All the isolates were absolutely resistant to benzylpenicillin 28(100%) – Table 3.

Table 1: Microbial identification using standard microbiological techniques

Tests	N	Positive n(%)	Negative n(%)
Catalase	28	28(100%)	0(0%)
Gram stain	28	24(85.71%)	4(14.29%)
Coagulase	28	25(89.29%)	3(10.71%)
Oxidase	28	4(14.29%)	24(85.71%)

Table 2: Susceptibility pattern of isolated microorganisms using agar disc diffusion methods

Antimicrobials	N	Susceptible n(%)	Resistant n(%)	Zone of Inhibition Mean ± SD
CPR	28	15 (53.57)	13 (46.43)	16.210 ± 2.215
OFL	28	13 (46.43)	15 (53.57)	14.235 ± 4.120
NIT	28	8 (28.57)	20 (71.43)	12.312 ± 1.022
AUG	28	10 (35.71)	18 (64.29)	12.674 ± 2.581
GEN	28	10 (35.71)	18 (64.29)	13.674 ± 3.131
CRX	28	5 (17.86)	23 (82.14)	10.846 ± 1.825

F-ratio=9.325, P-value <0.05
N-Number tested, CPR-Ciprofloxacin, OFL-Ofloxacin, GEN-Gentamycin, AUG-Amoxicillin/Clavulanate, NIT-Nitrofurantoin, CRX-Cefurozime

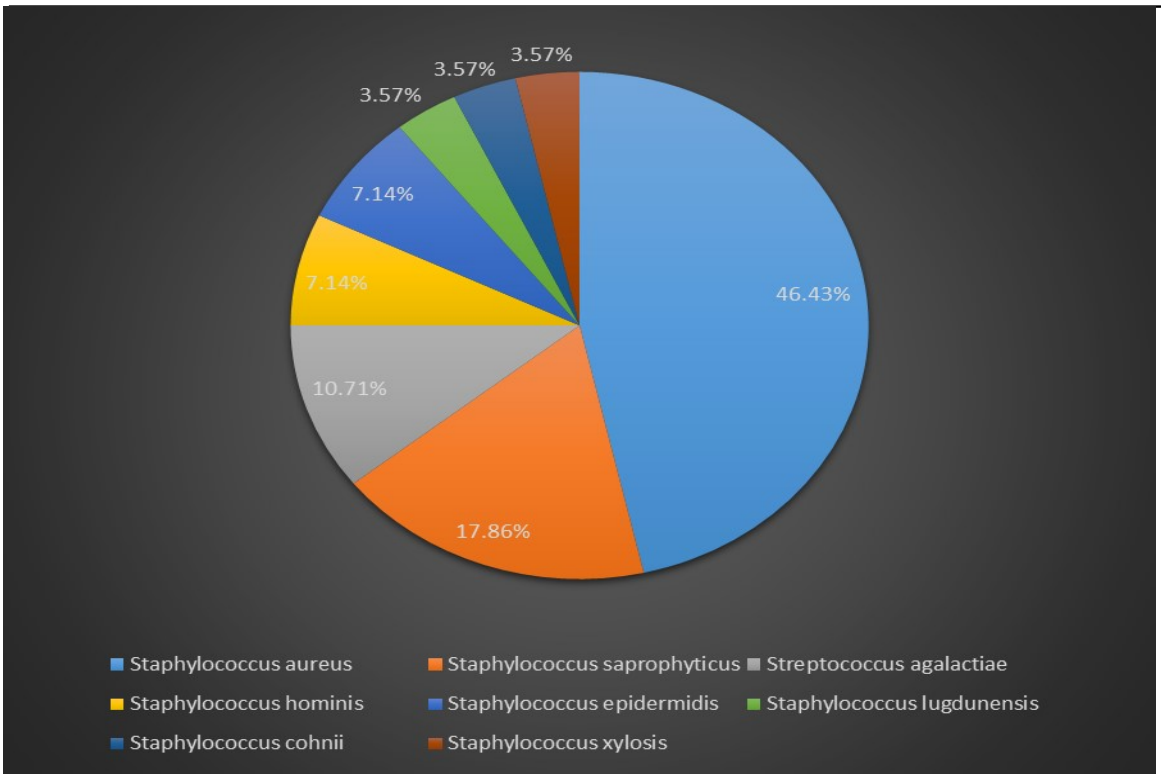

Fig.1: Frequency of Identified bacteria using Vitek-2

Table 3: Susceptibility pattern of isolated bacteria using Vitek-2 system

Antibiotics	**N**	**Sensitive n(%)**	**Intermediate n(%)**	**Resistance n(%)**
Oxacillin	28	10(35.71)	0(0.00)	18(64.29)
Gentamycin	28	21(75.00)	0(0.00)	7(25.00)
Trimethoprim/ Sulfamethoxazole	28	9(32.14)	0(0.00)	19(67.86)
Ciprofloxacin	28	24(85.71)	0(0.00)	4(14.29)
Erythromycin	28	11(39.29)	0(0.00)	17(60.71)
Vancomycin	28	13(46.43)	1(3.57)	14(50.00)
Tetracycline	28	15(53.57)	3(10.71)	10(35.71)
Nitrofurantoin	28	20(71.43)	4(14.29)	4(14.29)
Rifampicin	28	17(60.71)	0(0.00)	11(39.29)
Benzylpenicillin	28	0(0.00)	0(0.00)	28(100)

N- Number tested
n- Number sensitive, intermediate or resistant

DISCUSSION

The use of a rapid test that gives reliable and comprehensive results in microbial identification and susceptibility, has been proven to be clinically relevant. Out of the two hundred and seventy two skin swab samples collected, twenty eight were identified as Staphylococcus aureus based on the conventional methods. The identification process using the microbiological techniques has revealed that the bacteria isolates were mostly positive to gram stain test, coagulase test and they were all positive to catalase test. These results are suggestive of S. aureus which has been reported to exhibit these characteristics.

This is in agreement with the study of El-Hadedy and Salwa (2012) on the identification of Staphylococcus aureus and Escherichia coli isolated from Egyptian food by conventional and molecular methods, where they reported that the presumed S. aureus isolates were positive for coagulase, catalase, methylene red, Voges-proskauer and hemolysis tests but negative for oxidase and indole tests, thus, approved the isolates to be S. aureus. According to Smith *et al.* 2016, the gram staining test has been used to determine the type of bacteria by differentiating the chemical and physical properties of their respective cell walls. Moreover, the catalase test proves the presence of catalase enzymes in bacteria (Khatoon *et al.*, 2022).

The predominant identity of Staphylococcus aureus as revealed by Vitek-2 system, is in tandem with Mohammed *et al.* (2013), in the study of the incidence and antibiotic susceptibility pattern of bacterial isolates from wound infections in a tertiary hospital in Nigeria, where they reported S. aureus as the most predominant species among the wound bacterial contaminants. *S. aureus* is a normal flora of the body (Rahimi and Bouzari, 2015), however, it has been implicated in many nosocomial infections and has been responsible for both hospital and community -associated infections worldwide (Adesoji *et al.*, 2019). A study by Stanley *et al.* (2013), evaluated the Vitek-2 system diagnostic accuracy and concluded that the Vitek-2 system identified individual isolate to the species and strain level in order to establish the exact prevalence and antibiotic susceptibility.

Considering the importance of antimicrobial agents as first-line therapy for both mild and severe infections, this study revealed a notable difference in the level of accuracy of the antimicrobial susceptibility test (AST) methods employed. The Vitek-2 system analyzed a higher susceptibility of Staphylococcus spp to ciprofloxacin 24(85.71%) in contrast to the disc diffusion method 15(53.57%). This disparity might be attributed to the high sensitivity of the automated machine. The use of automated machine prevents error due to human interference thus ensuring an efficient and reliable output. Similarly, Kavipriya *et al.* (2021) studied the direct susceptibility test by Vitek-2 from positively flagged blood culture broth for gram-negative bacilli and reported that the Vitek-2 system demonstrated higher test accuracy, high performance for rapid AST and considerably reduced the turnaround time.

The higher susceptibility of Staphylococcus spp to ciprofloxacin agrees with the study of Ghali-Mohammed *et al.* (2023), where it was reported that a lower percentage of the isolated S. aureus demonstrated resistance to ciprofloxacin. This implies that ciprofloxacin could still be effective in the treatment of S. aureus induced infections within the studied population. The fluoroquinolones are commonly used antimicrobial agents in clinical

practice, however, the development of resistance to these agents by members of the staphylococci will further deplete the options available for chemotherapy (Stanley *et al*., 2013).

Ciprofloxacin is a bactericidal antibiotic agent (Fluoroquinolone) used in the treatment of bacterial infections such as urinary tract infections, pneumonia, skin infections, joint infections, gastrointestinal infections etc (Thai *et al*., 2023). This broad-spectrum quinolone antibiotic can be affordable and cost-effective as it is most potent against gram-negative bacilli bacteria and effective against some gram-positive bacteria. Hence, it inhibits DNA replication by inhibiting bacterial DNA topoisomerase and DNA-gyrase (Thai *et al*., 2023). It is noteworthy that the Staphylococcus spp exhibited multidrug resistance to trimethoprim, oxacillin, erythromycin and vancomycin respectively. The result of this study agrees with Adesoji *et al*. (2019) where they reported a high sensitivity of S. aureus to gentamycin but high resistance to erythromycin.

CONCLUSION

Staphylococcus aureus is the highest identified specie of the bacteria isolates from the collected skin swab samples by the Vitek-2 system. This study has demonstrated that ciprofloxacin exhibited the highest sensitivity against the bacteria and therefore discourages its abuse. Furthermore, the automated system displayed higher degree of accuracy in the identification of the bacteria to specie level and the antimicrobial susceptibility test. This study therefore conclude that the use of Vitek-2 system in clinical laboratories for routine identification and antimicrobial susceptibility of gram-positive cocci of medical relevance will be appropriate for adequate diagnosis of infection and accurate administration of treatment.

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