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MALDI-TOF MS Based Identification and Characterization of Staphylococci Isolates from Human and Animal Sources

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ABSTRACT

Identification of microorganisms usually depends on phenotypic typing and culture which may not give proper identification. The aim of this study is to use matrix-assisted desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) technique to accurately identify Staphylococci isolates from human and animal specimens; preliminarily identified by conventional microbiological techniques. Among the 112 preliminarily identified staphylococci isolates, MALDI-TOF MS showed that 23(20.5%) were *Staphylococcus aureus* while 89 (79.5%) were coagulase-negative staphylococci (CoNS). The identified CoNS were *Staphylococcus sciuri*, 56 (50.0%), *Staphylococcus haemolyticus* 7 (6.3%), *Staphylococcus cohnii* 5 (4.5%), *Staphylococcus arlettae* 1 (0.9%), *Staphylococcus simulans* 12 (10.7%), *Staphylococcus gallinarum* 6 (5.4%), *Staphylococcus lentus* 1 (0.9%) and *Staphylococcus piscifermentans* 1 (0.9%). *S. haemolyticus*, *S. cohnii* and *S. arlettae* were identified only from human specimens while *S. lentus* and *S. piscifermentans* were identified only from poultry specimens. All the identified staphylococci were susceptible to fosfomycin and imipenem, however, a very high rate of resistance was seen in oxacillin and benzyipenicillin; 82.6% and 95.7% for *S. aureus*; 61.8% and 78.7% for CoNS respectively. A total of 15(65.2%) of the identified *S. aureus* were positive for *mecA* gene while the 4(17.4%) that were PVL-positive did not harbour the *mecA* gene. The *spa* typing revealed that the stains were genetically diverse with the detection of nine different *spa* types from the 23 *S. aureus* strains. The findings from this study have shown that MALDI-TOF MS is a powerfully useful tool for accurate identification of Staphylococci to the species level.

***Keywords*:** MALDI-TOF MS, Staphylococci, Antibiotic Resistance, *spa* typing, Molecular characterization.

Introduction

Inadequate infrastructure, poor technical expertise and limited research capacity hampers the proper identification of bacteria in Sub-Saharan Africa. In Nigeria, bacterial identification is achieved mainly by cultural characteristics such as colonial morphology, Gram staining and biochemical tests including coagulase, catalase, indole, sugar fermentation etc. This time consuming and labour intensive approach limits the usefulness of the results when there is urgency for medical diagnosis.[1]

Staphylococcus aureus is a Gram-positive bacterium implicated in considerable human morbidity and mortality globally. Moreover, it colonizes economically important livestock species such as chicken, goat, cattle, pig etc. *S. aureus* is also the cause of mastitis in cattle. Colonized or infected animals may serve as a source of transmitting the pathogen to humans. The treatment and management of *S. aureus* infections are being challenged by the development of resistance to different class of antimicrobials in the pathogen; especially methicillin-resistant *S. aureus* (MRSA) which has become a major pathogen causing high morbidity and mortality worldwide.[2, 3]

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Coagulase-negative staphylococci (CoNS) are opportunistic pathogens that cause infection only when the natural immunity of the host is compromised such as incases of trauma or the implantation of medical devices.[4,5] The increasing rate of CoNS infections in Nigeria has necessitated the need for a robust method that can adequately identify the consortium of bacterial species comprising this group; as obtainable in the developed countries.[6, 7]

The matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) technique is a rapid, accurate and convenient tool for accurate identification of microorganisms with a high tendency of specificity and sensitivity.[8] Different biomedical investigators in the developed countries have used MALDI-TOF MS for the identification of different pathogens, detection of biological warfare agents and strain typing.[9-11] This technique provides a better alternative to in-depth molecular identification with comparable accuracy. Also, identification of microorganisms by MALDI-TOF MS is more cost-effective than by basic microbiological methods.[12] This has made it to be a powerful tool for adequate microbial identification and could replace conventional identification, but currently, many investigations using this technique have been mainly based in developed countries as there is a limited report on its application in Nigeria.[1, 13,14] Therefore, this study was designed to use MALDI-TOF MS to accurately identify staphylococcal isolates obtained from an ongoing antimicrobial-resistant surveillance study in southeast Nigeria and also carry out the molecular characterization of identified *S. aureus* isolates.

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Materials and Methods

Bacterial isolates and antibiotic susceptibility testing

The 112 staphylococcal isolates analyzed in this study were collected from specimens cultured as part of an antibiotics resistance surveillance study in southeast Nigeria. They were isolated from human clinical specimens and specimens from food animals viz: goats, cow, poultry and pigs. Preliminary identification of the staphylococcal isolates was based on colony morphology on mannitol salt agar and catalase tests. The isolates were then shipped to the Institute of Medical Microbiology and Hospital Hygiene, University of Dusseldorf, Germany for further studies. Susceptibility testing to Linezolid (10 µg), Tigecyclin (15 µg), Trimethoprim/sulfamethoxazole (25 µg), Erythromycin (15 µg), Clindamycin (2 µg), Cefazolin (5 µg), Gentamicin (10 µg), Levofloxacin (5µg), Moxifloxacin (5µg), Cefoxitin (30µg), Tetracycline (30µg), Ampicillin-sulbactam (20 µg), Oxacillin (1 µg), Cefotaxime (5 µg), Benzyipenicillin (1 µg) and Imipenem (10 µg), were determined using the modified Kirby-Bauer method on Muller Hinton agar. The European Committee on Antimicrobial Susceptibility Testing Guidelines was used to determine sensitivity and resistance.[15]

Identification using MALDI-TOF MS

A single colony of a fresh overnight culture of the presumably staphylococcal isolates was smeared on a MALDI-TOF target slide (BioMerieux, Germany), then overlaid with 2µl of matrix solution and air-dried at room temperature for 5 minutes. The matrix is a saturated solution of a- cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroace-tic acid. After the target preparation, it was then placed into the MALDI-TOF mass spectrometer system and the results were analyzed by MALDI Biotyper 3.0 software (Bruker Daltonik) following the manufacturer's instruction. *Escherichia coli* DH5 alpha laboratory strain was used as quality control on each experiment. The identification score cutoff value that was applied to each measurement was according to the manufacturer's instructions.[16]

DNA extraction

The extraction of genomic DNA was done using a DNeasy tissue kit (Qiagen, Hilden, Germany) with lysostaphin (100 µg/mL) to achieve bacterial lysis. A 2 mL fresh overnight culture of the staphylococcal isolates was used in each DNA extraction experiment.

Molecular characterization of identified S. aureus

The identified *S. aureus* strains were further characterized for genes encoding methicillin resistance (*mecA*), Panton-Valentine leukocidin (PVL), and staphylococcal protein A (*spa*). The *spa* typing of *S. aureus* strains was based on sequencing of the hypervariable region of the protein A gene (*spa*) as described by, [17] with the x region of the spa gene amplified by PCR. The Ridom StaphType software (Ridom GmbH, Wurzburg, Germany, version 2.1.1) was used to assign the *S. aureus spa* types. PCR assay was used to determine isolates encoding the PVL (virulence factor) and *mecA* (associated with methicillin resistance) genes respectively.[18]

Results and Discussion

Identification of Staphylococcal isolates

Among the 112 preliminarily identified Staphylococci isolates, MALDI-TOF MS showed that 23 (20.5%) were *Staphylococcus aureus* while 89 (79.5%) were coagulase-negative staphylococci (CoNS). The 112 isolates came from human and animal specimens; 48(42.9%) from human specimens, 18 (16.1%) from cow, 7 (6.25%) from goat, 17 (15.2%) from pig and 22 (19.6%) from poultry specimens. The identified CoNS were *Staphylococcus sciuri*, 56 (50.0%), *Staphylococcus haemolyticus* 7 (6.3%), *Staphylococcus cohnii* 5 (4.5%), *Staphylococcus arlettae* 1 (0.9%), *Staphylococcus simulans* 12 (10.7%), *Staphylococcus gallinarum* 6 (5.4%), *Staphylococcus lentus* 1 (0.9%) and *Staphylococcus piscifermentans* 1 (0.9%). *S. haemolyticus, S. cohnii* and *S. arlettae* were identified only from human specimens while *S. lentus* and *S. piscifermentans* were identified only from poultry specimens. *S. sciuri* and *S. simulans* were isolated from all sample sources, Table 1.

Antibiotic susceptibility testing

All the tested isolates were sensitive to fosfomycin and imipenem; similar trend of resistance was observed in *S. aureus* and CoNS, for example very high resistance was observed in oxacillin, cefotaxime and benzyipenicillin; 82.6%, 86.9 and 95.7 for *S. aureus*; 61.8%, 68.5% and 78.7% for CoNS respectively Figure 1 and Figure 2. All the identified *S. aureus* isolates apart from two strains G5 and Pgoat5 were multidrug resistance; five isolates were resistant to 10 different antibiotics Table 2.

Molecular characterization of identified S. aureus

Among the 23 identified *S. aureus*, 15(65.2%) were *mecA*-positive while 4(17.4%) were *PVL*-positive. All the *PVL*-positive isolates do not have the *mecA* gene, all the *S. aureus* strains that were *mecA*-positive exhibited MDR i.e. resistant to three or more antibiotic tested. Among the 15 MRSA isolates, 11 came from human specimens, while the remaining four came from poultry specimens; no MRSA strain was identified from goat, cow or pig specimens Table 3. The *spa* typing revealed that the stains were genetically diverse with the detection of nine different *spa* types from the 23 *S. aureus* strains. The most common *spa* type was t091 which was found only in human specimens; however, t024, t127, t442, t084, t105 and t318 were detected both from human and animal specimens. An overlap of *spa* types was observed between MSSA and MRSA strains Table 3.

In microbiology laboratories, the conventional identification of microorganisms is a time-consuming process that requires intensive labour. However, in recent years, a state-of-the-art mass spectrometry technique (MALDI-TOF) has emerged as a robust tool for accurate and fast identification of microorganisms and medical diagnosis. It has been successfully applied in the identification of a large number of microorganisms including yeasts, bacteria, and filamentous fungi.[19, 20, 21] It provides a better alternative to in-depth molecular identification with comparable accuracy. However, most biomedical researchers in Nigeria still face the challenge of proper identification of microorganisms in various laboratories across the country. They rely mainly on conventional phenotypic methods such as cultural characteristics, Gram staining and biochemical tests for microbial identification and characterization.[22] In most cases, these methods may not be very accurate, leading to false or improper identification of the microorganism; which may result to inappropriate treatment of patients.[14] There are very few published reports on the application of MALDI-TOF MS in the identification of microorganisms in Nigeria.[1,13,14] In this study, MALDI-TOS MS has allowed for accurate identification of staphylococci isolates from Nigeria that were initially poorly identified by the conventional techniques. The correct species identification was significantly higher than conventional cultural and biochemical methods (97.3% and 59.3%, respectively) and produced little or no incorrect genus identifications. Among the 112 preliminarily identified staphylococci isolates, MALDI-TOF MS showed that 23 (20.5%) were *Staphylococcus aureus* while 89 (79.5%) were coagulase-negative staphylococci (CoNS). The accuracy of identification observed in this study was similar to that reported by.[1, 23, 24] One of the presumed reasons limiting the application of this technique in microbiology laboratories in a developing country such as Nigeria is cost. However, some reports have shown that the application of MALDI-TOF MS technique in the identification of microorganisms comparatively reduced the costs for researchers in some developed countries.[25, 26, 27] Furthermore, [28] reported that when compared to the cost of different phenotypic tests usually carried out in developing countries to accurately identify a single microorganism; the cost for MALDI based identification may be cheaper. Nevertheless, the reliability and accuracy of results obtained by MALDI-TOF MS to allow for immediate therapeutic and clinical intervention are far more significant than that of the conventional method; though, it should be noted that the initial setup cost for this technique is expensive. The accurate identification of 97.3% of staphylococci tested in this study shows that MALDI-TOF MS is a powerful tool for bacterial species identification.

Considering antibiotics resistance, a similar trend of resistance was observed in *S. aureus* and CoNS isolates from human and animal specimens, for example very high resistance was observed in Oxacillin and Benzyipenicillin; 82.6% and 95.7 for *S. aureus*; 61.8% and 78.7%

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for CoNS respectively. The transmission of antibiotics resistant bacteria from animals to humans through the food chain or environment has been attributed to the use of antibiotics in veterinary medicine and for food animal production. In Nigeria, there is little or no regulation on the application of antibiotics in animal production, most farmers use antibiotics indiscriminately. Furthermore, the roles animals play as a reservoir of antibiotic-resistant traits which can be transferred to humans or a source of novel virulent zoonotic pathogens has been further emphasized by the emergence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA)**.** It is noteworthy to state that *S. aureus* and MRSA were identified from both human and animal specimens in this study, suggesting transmission of this strain among the two hosts. Therefore, the transfer of antibiotic-resistant bacteria from animals to humans through contacts or the food supply chain should be a cause for concern in public health. Also, the close contact of humans and animals either inform of pets or farm animals creates opportunities for the exchange of microbial flora and transmission of antibiotics resistant bacteria.[29, 30] It has been emphasized that people working in food animal production such as abattoir workers, farm labourers or veterinarians have more chances of being colonized with zoonotic pathogens than people that do not come in contact with animals. In addition to the proximity between human and animal hosts, the transfer of antibiotics-resistant traits may be achieved through different routes; such as movement of live animals (local, national and international), import and export of animal food products and travelling (local, national and international).[31] Nevertheless, not minding the route of transmission of antibiotics resistant microorganisms between humans and animals, the continued monitoring of bacterial populations between the two different hosts species should be encouraged for the health benefits of both. Therefore, through proper surveillance and monitoring, a potential route of transmission can be identified and appropriate policies and guidelines will be put in place to ensure that the efficacy of clinically important antibiotics is sustained.

Table 1: The Distribution of the Isolated Staphylococci among the Different Sample Sources

Source/ N (%)	*S. aureus* 23 (20.5)	*S. sciuri* 56 (50.0)	*S. haemolyticus* 7 (6.3)	*S. cohnii* 5 (4.5)	*S. arlettae* 1 (0.9)	*S. simulans* 12 (10.7)	*S. gallinarum* 6 (5.4)	*S. lentus* 1(0.9)	*S. piscifermentans* 1 (0.9)	Total N(%)
Human	17	17	7	5	1	1	-	-	-	48(42.9)
Cow	1	13	-	-	-	3	1	-	-	18(16.1)
Goat	1	3	-	-	-	1	2	-	-	7(6.3)
Pig	-	9	-	-	-	6	2	-	-	17(19.1)
Poultry	4	14	-	-	-	1	1	1	1	22(19.6)
Total	23	56	7	5	1	12	6	1	1	112

Table 2: The Antibiotics Resistance Profiles of the 23 *S. aureus* Identified by MALDI-TOF MS

S/N	Isolate Number	Sample source	Specimens	Antibiogram Profile	Total Drug Resistance
1	G5	Human	N. Swap	PG	1
2	Pgoat 5	Goat	N. Swab	PG,TS	2
3	36B	Human	Urine	CTX,PG,SAM	3
4	64	Human	W. swab	CTX,OX,PG, T	4
5	F35	Human	N. swab	CTX,OX,PG,SAM	4
6	H40	Human	N. swab	CTX,OX,PG,SAM	4
7	G22	Human	N. swab	CTX,OX,PG,TS, T	5
8	Poc36	Cow	N. swab	E,CL,T,LEV,MFX ,GN	6
9	G33	Human	N. swab	CTX,OX,PG,TS,SAM, E,CL	7
10	Ulow2	Poultry	Litter samples	CTX, OX,PG, T,LEV,MFX,GN	7
11	Uumr	Poultry	Litter samples	CTX, OX,PG,LEV,MFX,T,GN	7
12	H4	Human	N. swab	CTX,OX,PG,T, LEV,MFX,GN	7
13	H98	Human	N. swab	CTX, OX,PG,T, LEV,MFX,GN	7
14	H3	Human	N. swab	CTX, OX,PG, T,LEV,MFX, GN	7
15	Ulob4	Poultry	Litter sample	CTX, OX,PG,T, LEV,MFX,GN.TGS	8
16	F25	Human	N. swab	CTX,OX,PG,FOX,SAM, E,CL,T,CEF	9
17	G35	Human	N. swab	CTX,OX,PG,FOX,SAM, E,CL,T,CEF	9
18	H78	Human	N. swab	CTX, OX,PG,FOX,SAM, T ,LEV,MFX,CEF	9
19	36	Human	Urine	CTX,OX,PG,FOX, SAM,T, CEF,LEV,MFX,GN	10
20	28	Human	Urine	CTX,OX,PG,FOX,SAM, T ,LEV,MFX,CEF,GN	10
21	61	Human	HVS	CTX, OX,PG,FOX,SAM, T, LEV,MFX,CEF,GN	10
22	Ulof3	Poultry	Litter samples	CTX, OX,PG,FOX,SAM, T ,LEV,MFX,CEF,GN	10
23	42B	Human	Urine	CTX, OX,PG,FOX,SAM, T,LEV,MFX,CEF, LZD	10

* **KEY:** LZD = Linezolid, TGC = Tigecyclin, TS = Trimethoprim/sulfamethoxazole, E = Erythromycin, CL = Clindamycin, CEF = Cefazolin, GN = Gentamicin, LEV = Levofloxacin, MFX = Moxifloxacin, FOX = Cefoxitin, T = Tetracycline, SAM = Ampicillin-sulbactam, OX = Oxacillin, CTX = Cefotaxime, PG = Benzyipenicillin

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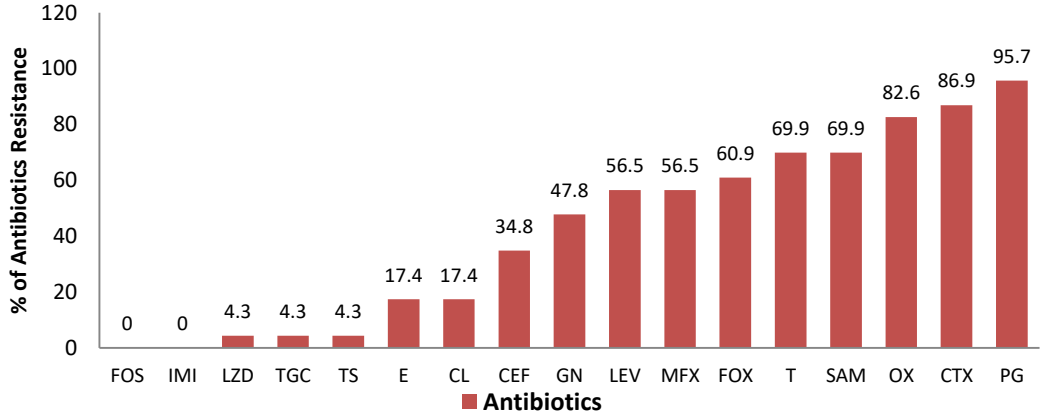

Figure 1: The Antibiotic Resistance Pattern of the 23 S. aureus Identified by MALDI-TOF MS

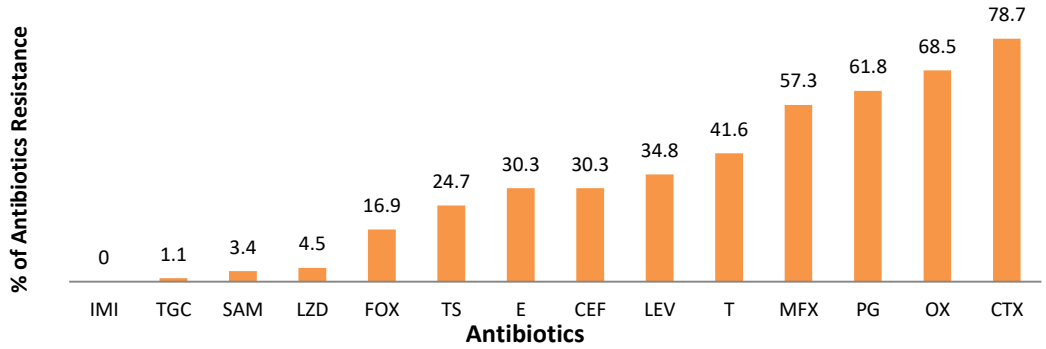

Figure 2: The Antibiotic Resistance Pattern of the 89 coagulase- negative Staphylococci Identified by MALDI-TOF MS

Table 3: Molecular Characteristics of the 23 *S. aureus* Identified by MALDI-TOF MS

S/N	Isolate Number	Sample origin	Molecular Characteristics		
			mecA	**PVL**	***spa type***
1	F25	Human	MRSA	PVL-ve	t786
2	G35	Human	MRSA	PVL-ve	t786
3	28	Human	MRSA	PVL-ve	t091
4	36	Human	MRSA	NA	NA
5	36B	Human	MSSA	PVL+ve	t355
6	G5	Human	MSSA	PVL+ve	t318
7	H4	Human	MRSA	PVL-ve	t105
8	64	Human	MRSA	PVL-ve	t024
9	G22	Human	MSSA	PVL-ve	t442
10	H3	Human	MRSA	NA	NA
11	42B	Human	MRSA	PVL-ve	t091
12	H78	Human	MRSA	PVL-ve	t091
13	G33	Human	MSSA	PVL+ve	t127
14	F35	Human	MSSA	PVL-ve	t355
15	H98	Human	MRSA	PVL-ve	t127
16	H40	Human	MSSA	PVL-ve	t084
17	61	Human	MRSA	PVL-ve	t091
18	Poc36	Cow	MSSA	PVL-ve	t105

Key: PVL +ve = 4, PVL –ve =17, NA =2

Conclusion

This study aligned with similar works done in developed countries; which showed that, to ensure adequate and proper bacterial identification, state-of-the-art identification technique such as MALDI-TOF MS should be applied. It is worthy to note that application of MALDI-TOF MS in microbiology laboratories for identification of microorganisms in Nigeria is possible, if relevant authorities will appreciate the importance of accurately identified bacterial strains and its impact on public health. Finally, there is *a* need for efficient and robust antimicrobial monitoring programmes in Nigeria to curtail the transmission of antibiotic-resistant traits from animals to humans and vice versa.

Conflict of Interest

All authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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