Chapter 4

Prevalence, Diagnosis and Local Susceptibility of Staphylococci Infections

Funmilola Abidemi Ayeni

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.74619

Abstract

Staphylococci are normally harmless commensals occurring on the skin, mucous membrane and the general environment. However, they are increasingly implicated in different infectious states. Of particular interest is the advent of methicillin-resistant *Staphylococcus aureus* (MRSA) with its attendance resistance to beta lactam antibiotics. Several infectious states are now emerging with staphylococci being implicated in the infections, e.g. *S. saprophyticus* has been implicated in urogenital infection. It would be interesting to document the prevalence of staphylococci in different infectious state. The identification of staphylococci is supposed to be a straightforward procedure, but an alarming misidentification rate is emerging in low resource laboratories, especially in places where identification is solely by growth and fermentation on mannitol salt agar (MSA). Finally, empirical treatment of any staphylococci infection will depend on local suspectibility pattern of the strains as the susceptibilities vary from environment to environment. This chapter summarizes the current knowledge regarding the prevalence, diagnosis and local susceptibility of staphylococci in different parts of the world.

Keywords: misdiagnosis, prevalence, susceptibility, staphylococci, antibiotics, identification

1. Introduction

Staphylococci have long history and association with mankind. From their presence in amniotic fluid, all through to adulthood, they were once regarded as harmless commensals with beneficial effects, e.g. by competing with pathogenic bacteria, but they are now implicated in life-threatening infections. Coagulase-negative staphylococci (CoNS) cause invasive infections in some vulnerable groups of patients, e.g. immunocompromised patients, preterm

neonates and people with indwelling medical devices [1]. *Staphylococcus epidermidis* is observed in 33% of blood samples collected from neonates, and it is the second most prevalent species observed in orthopedic device-related infections [2, 3]. *S. aureus* and *S. epidermidis* were ranked first in opportunistic infections and the major causative agent of medical implants and nosocomial infections in developing countries [4]. *S. epidermidis* was the commonest infectious species followed by *S. saprophyticus* and *S. haemolyticus* in a clinical study [5]. *S. lugdunensis* is implicated in infectious endocarditis. Although CoNS possess lesser virulence properties than *S. aureus*, they are more challenging due to their large proportion of methicillin-resistant strains with increasing numbers of isolates resistant to glycopeptides [6].

Most *S. aureus* infections used to be in the healthcare setting, but they are now established as a causative agent of serious infections in the community [7]. Immunocompromised individuals are at higher risk of general *S. aureus* infections (particularly invasive infection, e.g., bacteremia) than immunocompetent individuals. The emergence of community-acquired methicillin-resistant *S. aureus* (CA-MRSA) has led to an increase in the severity of infections of CA-MRSA in the last two decades [8]. Also, prevalence of methicillin-resistant coagulase-negative staphylococci has been reported, e.g. prevalence of nasal and pharyngeal carriage of methicillin-resistant *S. scuri* among 195 inpatients and healthcare workers in an healthcare center in Serbia has been reported as high in hospitals and possible dissemination across hospital wards can occur [9]. Several factors are responsible for transmission of *S. aureus* infections, e.g. domesticated animals in household transmission [7], neonates and breastfeeding mothers, immunocompromised patients, use of an indwelling intravascular plastic catheter, surgical incisions, open wounds, or burns.

The main aim of this chapter therefore is to highlight the current knowledge on prevalence, diagnosis and local susceptibility of staphylococci in different parts of the world.

2. Prevalence of staphylococci infections

S. aureus causes many infections including skin, soft tissue and invasive infections (complicated pneumonia, bacteremia, musculoskeletal infections and endocarditis). The common staphylococci infections are otitis media, bacteremia, skin infections, pneumonia, endocarditis, neonatal infections, osteomyelitis, food poisoning, toxic shock syndrome and scalded skin syndrome. However, there are increasing reports of staphylococcal infections in other parts of the body and vulnerable population.

2.1. Prevalence of staphylococci in different diseased condition

There are emerging facts on the role of staphylococci in central nervous system infections. In a multinational study performed with 2583 patients in 37 referral centers in 20 countries to understand the burden of community-acquired central nervous system infections between 2012 and 2014 [10], staphylococci and *Listeria* were responsible for frequent infections in immunocompromised patients. In another study of 102 patients on maintenance

hemodialysis, of 1402 patients hospitalized for infectious spondylodiscitis over a 13-year period, MRSA was the commonest pathogen found in the infectious sites followed by coagulase-negative staphylococci [11].

The role of staphylococci in burns and skin infections is well documented. In a retrospective study of 123 patients hospitalized in the burn center of Marrakech over a period of 3 years (2013–2016), there were 103 infections per 1000 days of treatment in different infective sites (blood (18%), skin (69%), lungs (1%) and urinary tract (12%)) with the main infectious organisms being: *Staphylococcus* sp. (37.7%) and MRSA in 22% of cases [12]. In Japan, a recent study investigated the antimicrobial resistance in pathogens isolated from skin and soft-tissue infections (SSTI) at 40 dermatology departments and clinics resulting in isolation of three main organisms (579 of *S. aureus* 141 (MRSA 24.4%), 240 of coagulase-negative staphylococci and 41 of *Streptococcus pyogenes*) identified from 860 strains [13].

Staphylococci are frequently implicated in hospital-acquired bacteremia especially those associated with intravascular catheters and staphylococcal bacteremia and they are important cause of morbidity. A study that described the epidemiology of healthcare-associated bloodstream infections for 71,039 patients in 338 Polish hospitals between 2012 and 2015 found that the most frequently isolated microorganisms were staphylococci (45.6%) and most of them were coagulase-negative (64.4%) and usually caused catheter-related infections. Of 53 *S. aureus* isolated, 24.5% were methicillin-resistant [14].

Immunocompromised patients are at higher risk of staphylococcal infections. In a study over a period of 1 year of *S. aureus* colonization, 81% of adults with human immunodeficiency virus (HIV) have higher rates of colonization than the general population [15]. Children with immunocompromising conditions are very vulnerable to *S. aureus* infections with the higher risk of development of complications in children with malignancy and high rates of resistance to antimicrobials [16].

Staphylococci are frequently implicated in neonatal infections usually within 6 week after birth with diseased conditions such as skin lesions, pneumonia, bacteremia, meningitis. In an epidemiology study of neonatal infection from 2005 to 2014 in 30 UK neonatal units, *E. coli* (15%), *S. aureus* (14%) and CoNS were prominent causes of late-onset sepsis in the neonates [17]. In sub-Saharan Africa, there is limited information on large-scale study on prevalence of staphylococci infections. However, Seale et al. [18] reported that from all neonatal admissions in a local hospital in Kenya from 1998 to 2013 to determine CoNS in neonates, CoNS was isolated from blood culture in 995 of 9552 (10%) neonates and the neonates with CoNS have higher risk of convulsions. Staphylococci were the most prevalent organism in a hospital-based case–control study in the Regional Hospital, Cameroon between September 2015 and August 2016 [19].

Otitis media is an inflammation of middle ear which may lead to hearing loss. *Streptococcus pneumoniae, Moraxella catarrhalis, Haemophilus influenzae* and *S. aureus* are some of the organisms implicated in acute otitis media. To assess the frequency of bacterial agents in 185 chronic suppurative otitis media, *Staphylococci* spp. (64.9%) were the most prevalent bacteria observed [20]. In some developing countries, otitis media may prevail as a result of illiteracy, poverty

and poor hygiene. In a study of 263 pus samples from 240 patients in a developing country, highest incidence of otitis media was observed in 1–10 year age group with the commonest bacteria isolated being *S. aureus* (36.11%) and CoNS (8.08%) [21].

2.2. MRSA prevalence in Africa

There is variable information on prevalence of MRSA in Africa. The prevalence was lower than 50% in most African countries and higher prevalence since 2000 has been observed in many African countries (except South Africa). In South Africa, the prevalence decreased between 2006 and 2011, while it varied between 23 and 44% for 2000–2007 in Botswana. It increased from 16 to 41% between 2002 and 2007 in Tunisia; in Libya, MRSA prevalence was 31% in 2007, while in Egypt and Algeria the prevalence was 45 and 52% between 2003 and 2005, respectively, while northern Nigerian had higher MRSA prevalence than the southern part with 55 and 39% prevalence in Ethiopia and Ivory Coast, respectively [22].

In a review of 34 reports from 15 countries in Africa, CC5 is the predominant clonal complex in healthcare setting in Africa. Hospital-associated MRSA was identified in nine African countries with limited spread of European ST80-IV clone to Algeria, Egypt and Tunisia and lack of distinct difference between MRSA responsible for hospital and community infections. However, the community clones (ST8-IV and ST88-IV) were observed in the hospital and community settings in Madagascar, Angola, Príncipe, Cameroon, Ghana, Gabon, Nigeria and São Tomé [23].

The overall prevalence of MRSA was 22.6% from 142 *S. aureus* isolates obtained from 261 samples sourced from university staff, students and fomites in Awka, Nigeria with the carriage rate being higher in females than male and highest in individuals of 20–30 years [24]. In a neonatal septicemia study involving 202 infants with risk factors for clinical features of septicemia in the first 3 days of life, 12.5% culture were positive with the predominant organisms being *S. aureus* (52%) and 30.7% being MRSA [25]. In intensive care unit of a Nigerian hospital, out of 71 patients with healthcare-associated infection, bloodstream and urinary tract infections were the commonest infections, and *S. aureus* was the commonest cause of bloodstream infection with 80% of the *S. aureus* being MRSA [26]. In surgical site infections for 103 patients with orthopedic surgery in a hospital in Port Harcourt, Nigeria, the commonest pathogen was *S. aureus* (34%) including 15 patients with MRSA [27]. and prevalence of MRSA with *mecA* gene as 42.3% from 156 *S. aureus* in clinical isolates from South Western Nigeria with domination of SCCmec II and SCCmec V [28].

A significant decline in antibiotic resistance was observed in Northeastern Nigeria in contrast to the worldwide trend of increasing resistance rates as stated in a study involving changes in population structure of *S. aureus* isolates in 2007 and 2012 from Northeastern Nigeria with a reduction in resistance to erythromycin, penicillin, clindamycin and gentamicin in 2012 with a decrease of MRSA [29]. The authors have confirmed low to moderate prevalence of MRSA in Nigeria in various studies. Ayeni et al. [30] reported 0.5% MRSA prevalence in nares of healthy adults. In another of our study by Ayeni et al. [31] where prevalence of MRSA in samples analyzed in Medical Microbiology Unit of University College Hospital, Ibadan between May and October 2012 was done. A 50 *S. aureus* strains were obtained with 34% of the studied *S. aureus* strains being phenotypically identified as MRSA strain.

3. Diagnosis of staphylococci infections

Identification of staphylococci is supposed to be a simple straightforward procedure that involves culturing of clinical specimens or pure bacterial strains on Columbia agar, mannitol salt agar (MSA) or tryptic soy blood agar. If pure biochemical identification is to be used, then Gram-positive, nonmotile, non-spore-forming, facultative anaerobic cocci occurring mainly in clusters and catalase-positive strains are selected for further tests. Coagulase test will distinguish between *S. aureus* and coagulase-negative staphylococci in clinical specimens. There is a current interest in small colony variant (SCV) of staphylococci which are nonhemolytic, nonpigmented and characterized by pinpoint colonies about 10% of the size of the normal colonies [32]. The SCV of *S. aureus* can contribute to persistent infection and they are associated with increased antibiotic resistance.

3.1. Overview of different staphylococci identification methods

Rapid latex and hemagglutination assays allows presumptive identification of *S. aureus* based on the detection of clumping factor, capsule types 5 and 8, protein A. They have high sensitivity (98–100%) and lower specificity (72–99%) which may be as a result of false-positive reactions occurring with some CoNS strains [6]. Novobiocin resistance is routinely used to distinguish the intrinsically resistant *S. saprophyticus* subsp. *saprophyticus* from other CoNS, e.g. *S. epidermidis* group. Tube coagulase test with horse plasma has been stated as a very accurate method to differentiate between *S. aureus* and CoNS. It has a high specificity [30].

There are commercial and automated systems for identification of staphylococci, e.g. Staphylococcus-specialized API Staph, Vitek 2, Rapidec Staph and ID32 Staph strips (bioMérieux) system, BBL Phoenix automated microbiology system, Crystal identification system's Rapid Gram-Positive ID kit (BD, MD), Pos ID Panel family (Siemens, Deerfield, IL), Sherlock microbial identification system (MIDI, Newark, DE) and the Biolog systems (Biolog, Hayward, CA) [6].

There are also several molecular approaches for identification of staphylococci. Conserved regions with species-specific sequences of universally occurring genes are amplified, for differentiation at the species level, e.g. 16S and 23S rRNA, *gap, gyrA, sodA, rpoB* and *tuf* genes. Sequencing of 16S rRNA gene has wide application in identification of bacterial species. However, other genes have been observed to be superior to 16S gene for identification of staphylococci. In a previous study, the author had also confirmed previous knowledge that sequencing of *tuf* gene has more discriminatory power in identification of staphylococci than partial sequencing of 16S rRNA gene [33]. Also, partial sequencing of *rpoB* gene has better identification power than partial 16S rRNA gene sequencing for the differentiation of *Staphylococcus* subspecies [34].

SCV strains grow on blood agar as pinpoint colonies and they are often nonreactive in normal biochemical tests because their laboratory detection could be affected by their altered metabolism and long generation time. Therefore, molecular methods, such as amplification of species-specific DNA targets or 16S rRNA partial sequencing, become the method of choice for their identification [35].

Microarray-based diagnostics test may combine identification of staphylococci with detection of virulence factors and drug resistance in strains. In positive blood culture smears, a nucleic acid hybridization assay (*S. aureus*/CNS PNA FISH; AdvanDx) targeting rRNA gene sequences with the principle of nucleic acid fluorescence in situ hybridization (PNA FISH) can be used for rapid identification of *S. aureus* [6]. Furthermore, spectroscopic and spectrometric methods, e.g. Fourier transform infrared (FTIR), Raman spectroscopy and MALDI-TOF MS are currently used in diagnostic laboratories. MALDI TOF has become a universal quick and accurate method for identification of microorganisms including staphylococci, and the principle is based on spectra obtained by molecular weight for individual fragments.

Identification of MRSA is primarily by cefoxitin disk screen test, the latex agglutination test for PBP2a or selective chromogenic agars [30]. Commercial tests are also available for identification of MRSA with combined detection of *mecA* and toxin genes. By using a multiplex PCR approach, *mecA* and *femA* can be simultaneously detected for rapid identification of MRSA and to differentiate *S. aureus* (*femA*1) from CoNS especially in blood samples [36]. An excellent correlation was reported between the broth microdilution assay and detection of antibiotic resistance genes by multiplex PCR [37].

In some institutions, there is active surveillance that uses rapid laboratory techniques to evaluate nasal swab specimens and routinely screen admitted patients, e.g. high-risk patients, patients with previous MRSA infection, vascular, orthopedic, or cardiac surgery patients for MRSA.

3.2. Wrong identification of staphylococci

Due to lack of adequate resources, wrong identification of *S. aureus* which could lead to wrong diagnosis of *S. aureus* infections has been observed. Mannitol salt agar (MSA) is often used in many laboratories in some developing countries, e.g. Nigeria for identification of *S. aureus*. The initial design of the agar was with the claim that it supports the growth of coagulase positive staphylococci only by being a selective and differential medium: The composition is 7.5% sodium chloride, mannitol as the carbohydrate and phenol red. It was claimed that colonies of CoNS and other salt-tolerant organisms will produce pink or red colonies, while *S. aureus* will grow on MSA as yellow colonies [38]. However, there are several evidences to disprove this claim.

One hundred and eight-five isolates that had been previously isolated from the nares of college students' volunteers in Southern Nigeria were identified by various methods. Growth on MSA and slide coagulase tests was highly inaccurate for identification of *S. aureus* although it is an indication of staphylococci; however, this should be taken with caution because other organisms like *Brevibacterium* can also grow on MSA with yellow colonies [30, 39]. The study confirmed that tube coagulase test with horse plasma, MALDI TOF mass spectrometry and PCR amplification of the *spa* gene are accurate diagnostic methods for identification of *S. aureus*. Also chromogenic medium, chromIDTM MRSA plate (bioMérieux, France) and Slidex MRSA Detection Kit (bioMérieux, France) were accurate for detection of MRSA [30].

In another study by Ayeni and Odumosu [40], it was noted that some organisms are being wrongly identified as *S. aureus* in phenotypic identifications. The study evaluated inaccurate identification of other organisms as *S. aureus* by collecting 507 phenotypically identified

S. aureus strains (identified by Gram staining, characteristic growth and fermentation on mannitol salt agar and blood agar and coagulase formation) obtained from 8 states in Southern Nigeria. Standard identification of the isolates was done in the study by sequencing of 16S rRNA gene and detection of *spa* gene. Fifty-four (11%) of the total isolates were confirmed as *S. aureus,* while the rest were CoNS with 85% misidentification, *Bacillus* sp. with 12% misidentification and *Brevibacterium* sp. with 3% misidentification. The study reported an alarming rate of false positive identification of *S. aureus* which could have resultant effect of misdiagnosis and subsequent wrong antibiotic prescription especially in emergency situation. Therefore, we demonstrated that CoNS grows and ferments mannitol on MSA. Standard methods should be used for identification of *S. aureus*.

We also studied 171 strains of CoNS which have been previously identified as *S. aureus* as a result of growth on MSA. The strains were collected from different locations in Nigeria, and ViTEK 2, MALDI-TOF MS and partial sequencing of 16S rRNA gene sequencing (gold standard) were used for identification. It was discovered that all strains (13 species of CoNS) grow on MSA and ferment mannitol. All tested strains of *S. warneri, S. epidermidis, S. pasteuri, S. sciuri, S. nepalensis, S. xylosus, S. capitis* and *S. haemolyticus* were correctly identified by MALDI-TOF, while all strains of *S. gallinarum* and *S. kloosii* were misidentified by MALDI TOF with total absence of *S. gallinarum* in the MALDI-TOF database at the period of this study. All tested strains of *S. warneri, S. epidermidis, S. xylosus S. gallinarum, S. sciuri, S. capitis* and *S. haemolyticus* were correctly identified by ViTEK, while the equipment misidentified *S. pasteuri* and *S. nepalensis*. It was concluded that growth on MSA for *S. aureus* is the same with CoNS and therefore the growth media cannot differentiate between CoNS and *S. aureus*. ViTEK seems more accurate than MALDI-TOF in identification of CoNS [33].

4. Susceptibility of staphylococci to antibiotics

As a basic principle, empiric use of antimicrobials should be guided by local epidemiology and antimicrobial susceptibility pattern as well as the clinical state of the patient, with final therapy determined by culture and sensitivity data. Vancomycin is the drug of choice for the treatment of MRSA infections, while clindamycin is the commonly used antimicrobial for CA-MRSA infections. However, many strains are emerging with reduced susceptibility to vancomycin for *S. aureus* and CoNS strains. MRSA are resistant to penicillin but susceptible to penicillinase-stable penicillins, such as methicillin and oxacillin. Healthcare-associated MRSAs are multiple resistant to other commonly used antimicrobial agents, including fluoroquinolones, erythromycin, tetracycline and clindamycin, while community-associated MRSAs are often resistant only to β-lactam agents and sometimes erythromycin and fluoroquinolones. In a study, 80% resistance to ampicillin was observed in CoNS, while resistance to cefoxitin and ceftriaxone was observed in 58% of the isolates [5].

In an antibiotic susceptibility study, 75.9% sensitivity to rifampicin, 100% sensitivity to vancomycin and linezolid was reported in catheter-related bloodstream infections in 58 (20 *S. aureus* and 38 CoNS) staphylococci in an Egyptian tertiary hospital with the recommendation

that linezolid and rifampicin could be used effectively against MRSA isolated from catheter-related bloodstream infections [41].

In another study on molecular epidemiology of trimethoprim resistance in 598 human *S. aureus* isolates collected in different locations across sub-Saharan Africa [Gabon, Nigeria (two), Namibia and Tanzania] [42]. About 54% of strains were observed to be resistant to trimethoprim and the resistance mostly mediated by *dfrG* gene, which is widespread in Africa. The study discourages the use of the drug for the treatment of SSTI caused by CA-MRSA.

Susceptibility of *S. aureus* strains to linezolid, rifampicin, teicoplanin, vancomycin, mupirocin phosphomycin, fusidic acid, daptomycin and tigecycline with 55 and 72% resistance to tetracycline and trimethoprim/sulphamethoxazole, respectively, has been reported, while in another study involving *S. aureus* isolates obtained from infection and asymptomatic carriers in Lagos and Ogun States, Nigeria, higher resistance was observed for aminoglycosides in clinical isolates, and more prevalent resistances to quinolones and tetracycline were observed in carrier isolates [37, 43].

Daptomycin and quinupristin/dalfopristin have been proposed as an alternative to glycopeptides in the treatment of MRSA infections, while the use of telithromycin is discouraged [44]. Also, all MRSAs were sensitive to amikacin, ciprofloxacin and chloramphenicol, while all methicillin-sensitive *S. aureus* were sensitive to ampicillin/sulbactam in a study [25], while fusidic acid resistance was reported in 93.7% of isolates, from prevalence of nasal and pharyngeal carriage of MRSA among inpatients and healthcare workers in an healthcare center in Serbia [9].

Osteomyelitis occurs more frequently in children, causing pains, chills and fever. Osteomyelitis regularly involves prolonged systemic antibiotic use, and dalbavancin, linezolid and vancomycin were active against staphylococci implicated in bone and joint infections [45].

All *S. aureus* strains in otitis media case in a developing country were sensitive to gentamycin [21], while ciprofloxacin was stated as the most effective antibiotic for treatment of bacterial chronic suppurative otitis media [20]. In a study on the prevalence and antimicrobial susceptibility pattern of external ocular bacterial infections in Ethiopia, the prevalence of MRSA infection was 24%, and multidrug resistance was observed in 87% of the isolated bacteria [46].

Ayeni et al. [30] reported susceptible to fusidic acid, rifampicin clindamycin, vancomycin and linezolid, with observed high resistance to penicillin and trimethoprim in 185 staphylococci, which had been previously isolated from the nares of college students' volunteers in Southern Nigeria. In another study by Ayeni et al. [31] where the current resistant pattern of *S. aureus* to β lactam antibiotics in samples analyzed in Medical Microbiology Unit of University College Hospital, Ibadan between May and October 2012 were evaluated. A 50 *S. aureus* strains were obtained which were highly resistant to erythromycin (72%), clindamycin (78%), aztreonam (70%) and amoxycillin (92%), but highly susceptible to imipenem (90%). Variable resistance was observed to cefotaxime (62%), ceftazidine (50%), cefoxitin (66%), ceftriazone (52%) and amoxycillin/clavulanic acid (50%). All the isolates resistant to amoxicillin/clavulanic were, however, susceptible to ≥1 of the cephalosporins. All phenotypic identified MRSAs were resistant to amoxicillin, erythromycin, clindamycin amoxicillin/clavulanic acid

and ≥1 cephalosporin (except 1). About 88% of the studied MRSA strains were sensitive to imipenem. *S. aureus* strains (42%) susceptible to amoxicillin/clavulanic acid were resistant to amoxicillin. A synergy was observed between imipenem and aztreonam in some isolates which were resistant to aztreonam but sensitive to imipenem which may be an indication that combined therapy of imipenem and aztreonam may result in enhanced antimicrobial activity of aztreonam. We concluded that cephalosporins are still relatively effective for treatment of *S. aureus* infections due to in vitro evidence.

Another study in our group determined antimicrobial resistance of staphylococci isolated from urogenital tracts of humans with a presumptive diagnosis of urinary tract infection in 45 urogenital samples (endocervical swab, high vaginal swab and urine) from outpatients at Igbinedion University Teaching Hospital between April and May 2010. Ten isolates (22% of the total samples) of staphylococci were obtained. All the isolates were multidrug resistant with exhibited resistance to ≥5 antimicrobials and 100% resistance to ciprofloxacin, nitrofurantoin, augmentin, ampicillin and ceftriazone. All CoNS strains were susceptible to doxycycline, while *S. aureus* strains were relatively susceptible to TMP/SMX [47].

Ceftobiprole and ceftaroline are new cephalosporins active against *S. aureus*, including MRSA strains causing infections like pneumonia and staphylococci soft tissue infections in adults. They have been recently approved in Europe (Ceftobiprole by the European Medicines Agency) and the USA (Ceftaroline by U.S. Food and Drug Administration) for treatment of *S. aureus* and MRSA infections. However, resistance to these antibiotics is emerging, and it is often associated with mutations in *mecA,* increasing in the production of PBP4, which mediates resistance to ceftobiprole and ceftaroline [48]. However, a global surveillance conducted prior to the European launch of ceftaroline revealed 4 *S. aureus* from 8037 tested strains with ceftaroline resistance [49]. In another study on ceftaroline against 1971 *S. aureus* isolates collected from seven countries in the Asia-Pacific region in 2012 [50], there was ceftaroline susceptibility rate of 86.9%, and surprisingly in Thailand, more than half (52.8%) of isolates were resistant to ceftaroline. Minimal resistance to ceftobiprole has been reported by Hodille et al. [51] (1 of 440 *S. aureus*) strains isolated from bronchopulmonary infections being resistant to ceftobiprole, while another study involving MRSA isolates from colonization (n = 37) and infection (n = 23) isolated from Côte d'Ivoire, Congo, Gabon and Nigeria, 16.7 and 15% of strains were resistant to ceftaroline and ceftobiprole, respectively, and surprisingly detected only in Nigeria [52].

4.1. Susceptibility of staphylococci to non-antibiotic substances

Other natural and beneficial bacteria have been found to be effective against staphylococci in vitro. This has been demonstrated in previous studies. The first discussion is on medicinal plants that have been proven over many generations to be effective against several infectious diseases. The plants from the genus *Combretum* have been shown to be part of recipe for the traditional treatment of various diseases with broad antimicrobial spectrum of 36 species of the genus having antimicrobial activities [53, 54]. In our study involving the antibacterial activities of the methanol extracts from the leaves of *Combretum hispidum*, *Combretum racemosum* and *Combretum platypterum* against seven strains of MRSA in vitro, extract from

Combretum racemosum leaves had high anti-MRSA activities (0.16–1.25 mg/mL MIC values) on all tested strains of MRSA [55]. This could be a potential source of newer antimicrobial agent against MRSA infections.

Lactic acid bacteria (LAB) are beneficial bacteria with good antimicrobial activities against many pathogenic bacteria. We reported good inhibition of growth of uropathogenic *S. saprophyticus* and *S. aureus by L. fermentum, L. brevis, L. plantarum, Streptococcus durans* and *Lactococcus lactis* [56]. In another study in our group, LAB from salad vegetables have anti-MRSA abilities in vitro against five confirmed MRSA strains. *P. pentosaceus* and *L. cellobiosus* exhibited widest zones of inhibition. In the agar overlay, *P. pentosaceus* and *W. confusa* showed the widest zones of 28 and 24 mm, respectively [57].

In a recently published co-culture study that we did with LAB and MRSA, the cell free supernatant of *L. fermentum* and *L. plantarum* was generally active against MRSA, the largest zone of inhibition was 13 mm with *L. plantarum*. Further experiment of co-culture was done in the study with MRSA and *L. plantarum* 9, *L. buchneri* SM04, *L. fermentum* 008, *L. brevis* 21 and *Weissella paramesenteroides*. All the tested LAB reduce the viable counts of MRSA from 10 log to 3 log after 24 h of co-incubation, while *L. plantarum* 9 and *L. fermentum* 008 totally inhibited the growth of MRSA after 72 h of co-culture and the MRSA could not grow in overnight culture of LAB, after 24 h of incubation [58]. In another study from our group, LAB strains were assayed for antimicrobial ability against two uropathogenic *S. aureus* strains. The selected *Staphylococcus* spp. was generally resistant to macrolides (100% resistance to clarithromycin), aminoglycosides (50–90%), fosfomycin and rifampicin (20%), while 70% resistance was observed in co-trimoxazole. Lesser resistance was observed in the quinolones (10–20%), and β lactams antibiotics have variable resistance (30–90%). However, 22 LAB strains had strong suppression of target *S. aureus* strains with clear zones (>10 mm) around the streaks [59].

5. Conclusion

Staphylococci are implicated in various infectious states in different parts of the world with high prevalence. However, characteristics growth on mannitol salt agar is insufficient to differentiate between *S. aureus* and coagulase-negative staphylococci. Other standard identification methods should be used. Staphylococci antibiotic susceptibility varies between different locations and site of infection.

Conflict of interest

The author declares that there is no conflict of interest.

Author details

Funmilola Abidemi Ayeni

Address all correspondence to: funmiyeni@yahoo.co.uk

Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria

References

[1] Zanzoni A, Montecchi-Palazzi L, Quondam MX. A molecular interaction database. FEBS Letters. 2002;**513**:135-140. DOI: 10.1016/s0014-5793(01)03293-8

[2] Brescó MS, Harris LG, Thompson K, Stanic B, Morgenstern M, O'Mahony L, Richards RG, Moriarty TF. Pathogenic mechanisms and host interactions in *Staphylococcus epidermidis* device-related infection. Frontiers in Microbiology. 2017;**8**:1401

[3] Kohli-Kochhar R, Omuse G, Revathi G. A ten year review of neonatal bloodstream infections in a tertiary private hospital in Kenya. Journal of Infection in Developing Countries. 2011;**5**:799-803

[4] Chessa D, Ganau G, Mazzarello V. An overview of *Staphylococcus epidermidis* and *Staphylococcus aureus* with a focus on developing countries. Journal of Infection in Developing Countries. 2015;**9**(6):547-550

[5] Shrestha LB, Bhattarai NR, Khanal B. Antibiotic resistance and biofilm formation among coagulase-negative staphylococci isolated from clinical samples at a tertiary care hospital of eastern Nepal. Antimicrobial Resistance and Infection Control. 2017;**6**:89. DOI: 10.1186/s13756-017-0251-7. eCollection 2017

[6] Becker K, Heilmann C, Peters G. Coagulase-negative Staphylococci. Clinical Microbiology Reviews 2014;(4):870-926

[7] Knox J, Uhlemann A, Lowy FD. Staphylococcus aureus infections: Transmission within households and the community. Trends in Microbiology. 2015;**23**(7):437-444

[8] Dukic VM, Lauderdale DS, Wilder J, Daum RS, David MZ. Epidemics of community-associated methicillin-resistant Staphylococcus aureus in the United States: A meta-analysis. PLoS One. 2013;**8**(1):e52722

[9] Cirkovic I, Trajkovic J, Hauschild T, Andersen PS, Shittu A, Larsen AR. Nasal and pharyngeal carriage of methicillin-resistant Staphylococcus sciuri among hospitalised patients and healthcare workers in a Serbian university hospital. PLoS One. 2017;**12**(9):e0185181. DOI: 10.1371/journal.pone.0185181

[10] Erdem H, Inan A, Guven E, Hargreaves S, Larsen L, Shehata G, Pernicova E, Khan E, Bastakova L, Namani S, et al. The burden and epidemiology of community-acquired central nervous system infections: A multinational study. European Journal of Clinical Microbiology & Infectious Diseases. 2017;**36**(9):1595-1611. DOI: 10.1007/s10096-017-2973-0. Epub 2017 Apr 10

[11] Lu YA, Sun WC, Kuo G, Chen CY, Kao HK, Lin Y, Lee CH, Hung CC, Tian YC, Ko YS, Hsu HH. Epidemiology and outcomes of infectious spondylodiscitis in hemodialysis patients. Spine (Phila Pa 1976). 2017 Oct 9. DOI: 10.1097/BRS.0000000000002443

[12] Benchamkha Y, Dhaidah O, Dahazze A, Meriem Q, Elamrani MD, Ettalbi S. The bacteriological profile of the burned patients in the center of burns in CHU Mohamed VI Marrakech (about 123 cases). International Journal of Burns and Trauma. 2017;**7**(6):72-79

[13] Watanabe S, Ohnishi T, Yuasa A, Kiyota H, Iwata S, Kaku M, Watanabe A, Sato J, Hanaki H, Manabe M, et al. The first nationwide surveillance of antibacterial susceptibility patterns of pathogens isolated from skin and soft-tissue infections in dermatology departments in Japan. Journal of Infection and Chemotherapy. 2017;**8**:503-511. DOI: 10.1016/j.jiac.2017.05.006

[14] Deptuła A, Trejnowska E, Dubiel G, Wanke-Rytt M, Deptuła M, Hryniewicz W. Healthcare associated bloodstream infections in Polish hospitals: prevalence, epidemiology and microbiology—Summary data from the ECDC Point Prevalence Survey of Healthcare Associated Infections 2012-2015. European Journal of Clinical Microbiology & Infectious Diseases. 2017. DOI: 10.1007/s10096-017-3150-1

[15] Gordon RJ, Chez N, Jia H, et al. The NOSE study (nasal ointment for Staphylococcus aureus eradication): A randomized controlled trial of monthly mupirocin in HIV-infected individuals. Journal of Acquired Immune Deficiency Syndromes. 2010;**55**(4):466-472

[16] McNeil JC. Staphylococcus aureus—Antimicrobial resistance and the immunocompromised child. Infection and Drug Resistance. 2014;**7**:117-127

[17] Cailes B, Kortsalioudaki C, Buttery J, Pattnayak S, Greenough A, Matthes J, Bedford Russell A, Kennea N, Heath PT, neonIN network. Epidemiology of UK neonatal infections: The neonIN infection surveillance network. Archives of Diseases in Childhood. Fetal and Neonatal Edition. 2017. pii: fetalneonatal-2017-313203. DOI: 10.1136/archdischild-2017-313203

[18] Seale AC, Obiero CW, Jones KD, Barsosio HC, Thitiri J, Ngari M, Morpeth S, Mohammed S, Fegan G, Mturi N, Berkley JA. Should first-line empiric treatment strategies for neonates cover coagulase-negative staphylococcal infections in Kenya? The Pediatric Infectious Disease Journal. 2017;(11):1073-1078. DOI: 10.1097/INF.0000000000001699

[19] Njim T, Aminde LN, Agbor VN, Toukam LD, Kashaf SS, Ohuma EO. Risk factors of lower limb cellulitis in a level-two healthcare facility in Cameroon: A case-control study. BMC Infectious Diseases. 2017;**17**(1):418. DOI: 10.1186/s12879-017-2519-1

[20] Mofatteh MR, Shahabian Moghaddam F, Yousefi M, Namaei MH. A study of bacterial pathogens and antibiotic susceptibility patterns in chronic suppurative otitis media. The Journal of Laryngology and Otology. 2018;**132**(1):41-45. DOI: 10.1017/S0022215117002249

[21] Basnet R, Sharma S, Rana JC, Shah PK. Bacteriological study of otitis media and its antibiotic susceptibility pattern. Journal of Nepal Health Research Council. 2017;**15**(2):124-129

[22] Falagas ME, Karageorgopoulos DE, Leptidis J, Korbila IP. MRSA in Africa: Filling the global map of antimicrobial resistance. PLoS One. 2013 Jul 29;**8**(7):e68024. DOI: 10.1371/journal.pone.0068024

[23] Abdulgader SM, Shittu AO, Nicol MP, Kaba M. Molecular epidemiology of methicillin-resistant Staphylococcus aureus in Africa: A systematic review. Frontiers in Microbiology. 2015;**6**:348. DOI: 10.3389/fmicb.2015.00348. eCollection 2015

[24] Ike B, Ugwu MC, Ikegbunam MN, Nwobodo D, Ejikeugwu C, Gugu T, Esimone CO. Prevalence, antibiogram and molecular characterization of comunity-acquired methicillin-resistant *Staphylococcus Aureus* in AWKA, Anambra Nigeria. Open Microbiology Journal. 2016;**10**:211-221. DOI: 10.2174/1874285801610010211

[25] Akindolire AE, Tongo O, Dada-Adegbola H, Akinyinka O. Etiology of early onset septicemia among neonates at the University College Hospital, Ibadan, Nigeria. Journal of Infection in Developing Countries. 2016;**10**(12):1338-1344. DOI: 10.3855/jidc.7830

[26] Iwuafor AA, Ogunsola FT, Oladele RO, Oduyebo OO, Desalu I, Egwuatu CC, Nnachi AU, Akujobi CN, Ita IO, Ogban GI. Incidence, clinical outcome and risk factors of intensive care unit infections in the Lagos University Teaching Hospital (LUTH), Lagos, Nigeria. PLoS One. 2016;**11**(10):e0165242. DOI: 10.1371/journal.pone.0165242

[27] Murphy RA, Okoli O, Essien I, Teicher C, Elder G, Pena J, Ronat JB, Bernabé KJ. Multidrug-resistant surgical site infections in a humanitarian surgery project. Epidemiology and Infection. 2016;**11**:1-7

[28] Alli OA, Ogbolu DO, Shittu AO, Okorie AN, Akinola JO, Daniel JB. Association of virulence genes with mecA gene in Staphylococcus aureus isolates from Tertiary Hospitals in Nigeria. Indian Journal of Pathology and Microbiology. 2015;**58**(4):464-471. DOI: 10.4103/0377-4929.168875

[29] Okon KO, Shittu AO, Kudi AA, Umar H, Becker K, Schaumburg F. Population dynamics of *Staphylococcus aureus* from Northeastern Nigeria in 2007 and 2012. Epidemiology and Infection. 2014;**142**(8):1737-1740. DOI: 10.1017/S0950268813003117

[30] Ayeni FA, Gbarabon TB, Andersen C, Nørskov-Lauritsen N. Comparism of identification and antimicrobial resistance pattern of Staphylococcus aureus isolated from Amassoma, Bayelsa State, Nigeria. African Health Sciences. 2015;**15**(4):1282-1288

[31] Ayeni FA, Olatunji DF, Ogunniran M. Prevalence of methicillin resistant Staphylococcus aureus and resistance pattern of its clinical strains to beta-lactam antibiotics. African Journal of Biomedical Research. 2014;**17**:129-133

[32] Proctor RA, von Eiff C, Kahl BC, Becker K, McNamara P, Herrmann M, Peters G. Small colony variants: A pathogenic form of bacteria that facilitates persistent and recurrent infections. Nature Reviews. Microbiology. 2006;**4**:295-305

[33] Ayeni FA, Andersen C, Nørskov-Lauritsen N. Comparison of growth on mannitol salt agar, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, VITEK® 2 with partial sequencing of 16S rRNA gene for identification of coagulase-negative staphylococci. Microbial Pathogenesis. 2017;**105**:255-259

[34] Mellmann A, Becker K, von Eiff C, Keckevoet U, Schumann P, Harmsen D. Sequencing and staphylococci identification. Emerging Infectious Diseases. 2006;**12**:333-336

[35] Melter O, Radojevič B. Small colony variants of *Staphylococcus aureus*—Review. Folia Microbiologia (Praha). 2010;**55**(6):548-558. DOI: 10.1007/s12223-010-0089-3

[36] Vannuffel P, Gigi J, Ezzedine H, Vandercam B, Delmee M, Wauters G, Gala J. Specific detection of methicillin-resistant *Staphylococcus* species by multiplex PCR. Journal of Clinical Microbiology. 1995;**33**(11):2864-2867

[37] Shittu AO, Okon K, Adesida S, Oyedara O, Witte W, Strommenger B, Layer F, Nübel U. Antibiotic resistance and molecular epidemiology of *Staphylococcus aureus* in Nigeria. BMC Microbiology. 2011;**11**:92. DOI: 10.1186/1471-2180-11-92

[38] Dugud JP. Staphylococcus: Cluster-forming gram-positive cocci. In: Colle JG, Duguid JP, Fraser AG, Marmion BP, editors. Mackie and McCartney Practical Medical Microbiology. 13th ed. New York: Churchill Livingstone; 1989. pp. 303-316

[39] Ayeni FA, Okwu M. Comparison of ViTEK 2, MALDI-TOF and partial sequencing of 16S rRNA gene in identification of Brevibacterium species with its antibiotic susceptibility pattern. Nigerian Journal of Pharmaceutical Research. 2016;**12**(1):46-51

[40] Ayeni FA, Odumosu BT. False identification of other microorganisms as Staphylococcus aureus in Southern Nigeria. Tropical Journal of Pharmaceutical Research. 2016;**15**(9): 1941-1945

[41] Hashem AA, Abd E, Fadeal NM, Shehata AS. In vitro activities of vancomycin and linezolid against biofilm-producing methicillin-resistant Staphylococci species isolated from catheter-related bloodstream infections from an Egyptian tertiary hospital. Journal of Medical Microbiology. 2017;**66**(6):744-752

[42] Nurjadi D, Olalekan AO, Layer F, Shittu AO, Alabi A, Ghebremedhin B, Schaumburg F, Hofmann-Eifler J, Van Genderen PJ, Caumes E, Fleck R, Mockenhaupt FP, Herrmann M, Kern WV, Abdulla S, Grobusch MP, Kremsner PG, Wolz C, Zanger P. Emergence of trimethoprim resistance gene dfrG in Staphylococcus aureus causing human infection and colonization in sub-Saharan Africa and its import to Europe. The Journal of Antimicrobial Chemotherapy. 2014;**69**(9):2361-2368. DOI: 10.1093/jac/dku174

[43] Ayepola OO, Olasupo NA, Egwari LO, Becker K, Schaumburg F. Molecular characterization and antimicrobial susceptibility of Staphylococcus aureus isolates from

clinical infection and asymptomatic carriers in Southwest Nigeria. The PLoS One. 2015;**10**(9):e0137531. DOI: 10.1371/journal.pone.0137531

[44] Oksuz L, Gurler N. Susceptibility of clinical methicillin-resistant Staphylococci isolates to new antibiotics. Journal of Infection in Developing Countries. 2013;**7**(11):825-31. DOI: 10.3855/jidc.3867

[45] Pfaller MA, Flamm RK, Castanheira M, Sader HS, Mendes RE. Dalbavancin in vitro activity obtained against gram-positive clinical isolates causing bone and joint infections in United States and european hospitals (2011-2016). International Journal of Antimicrobial Agents. 2017. pii: S0924-8579(17)30445-4. DOI: 10.1016/j.ijantimicag.2017.12.011

[46] Getahun E, Gelaw B, Assefa A, Assefa Y, Amsalu A. Bacterial pathogens associated with external ocular infections alongside eminent proportion of multidrug resistant isolates at the University of Gondar Hospital, northwest Ethiopia. BMC Ophthalmology. 2017;**17**(1):151. DOI: 10.1186/s12886-017-0548-6

[47] Ayeni FA, Omoregie O, Olley M. Resistance pattern of uropathogenic staphylococcal strains isolated from outpatients in a Nigerian Hospital. Journal of Medical and Applied Biosciences. 2010;**2**:38-45

[48] Hamilton SM, Alexander AN, Choo EJ, Basuino L, da Costa TM, Severin A, Chung M, Aedo S, Strynadk NCI, Tomasz A, Chatterjee SS, Chamber HF. High-level resistance of Staphylococcus aureus to β-lactam antibiotics mediated by penicillin-binding protein 4 (PBP4). Antimicrobial Agents and Chemotherapy. 2017;**61**(6):e02727-e02716

[49] Alm RA, McLaughlin RE, Kos VN, Sader HS, Iaconis JP, Lahiri SD. Analysis of Staphylococcus aureus clinical isolates with reduced susceptibility to ceftaroline: An epidemiological and structural perspective. The Journal of Antimicrobial Chemotherapy. 2014;**69**(8):2065-2075

[50] Biedenbach DJ, Alm RA, Lahiri SD, Reiszner E, Hoban DJ, Sahm DF, Bouchillon SK, Ambler JE. In vitro activity of ceftaroline against *Staphylococcus aureus* isolated in 2012 from Asia-Pacific countries as part of the AWARE Surveillance Program. Antimicrobial Agents and Chemotherapy. 2015;**60**(1):343-7

[51] Hodille E, Delouere L, Bouveyron C, Meugnier H, Bes M, Tristan A, Laurent F, F3 V, Lina G, Dumitrescu O. In vitro activity of ceftobiprole on 440 *Staphylococcus aureus* strains isolated from bronchopulmonary infections. Médecine et Maladies Infectieuses. 2017;**47**(2):152-157

[52] Schaumburg F, Peters G, Alabi A, Becker K, Idelevich EA. Missense mutations of PBP2a are associated with reduced susceptibility to ceftaroline and ceftobiprole in African MRSA. The Journal of Antimicrobial Chemotherapy. 2016;**71**(1):41-44

[53] Ogbole OO. Mutiu W, Osungunna MO. Effect of three Nigerian medicinal plants on methicillin resistant *Staphylococcus aureus* (MRSA). Ethnopharmacology. 2011;**2**

[54] de Morais Lima GR, de Sales IR, Caldas Filho MR, de Jesus NZ, de Sousa FH, Barbosa-Filho JM. Bioactivities of the genus *Combretum* (Combretaceae): A review. Molecules. 2012;**17**:9142-9206

[55] Ogbole OO, Ayeni FA, Ajaiyeoba EO. In-vitro antibacterial screening of methanol extracts of three *Combretum* species against seven strains of methicillin resistant *Staphylococcus aureus* (MRSA). Nigerian Journal of Pharmaceutical Research. 2016;**12**(2):149-154

[56] Adeniyi BA, Ayeni FA, Ogunbanwo ST. Antagonistic activities of lactic acid bacteria isolated from Nigerian fermented dairy foods against organisms implicated in urinary tract infection. Biotechnology. 2006;**5**(2):183-188

[57] Bamidele TA, Adeniyi BA, Ayeni FA, Fowora MA, Smith SI. The antagonistic activities of lactic acid bacteria isolated from Nigerian salad vegetables against methicillin resistant Staphylococcus aureus. Global Research Journal of Microbiology. 2013;**3**(1):18-23

[58] Alebiosu KM, Adetoye A, Ayeni FA. Antimicrobial activities of lactic acid bacteria against *Pseudomonas aeruginosa, Providencia vermicola, Alcaligenes faecalis* and methicillin resistant *S. aureus*. West African Journal of Pharmacy. 2017;**28**(2):132-142

[59] Ayeni FA, Adeniyi BA, Ogunbanwo ST, Tabasco R, Paarup T, Peláez C, Requena T. Inhibition of uropathogens by lactic acid bacteria isolated from dairy foods and cow's intestine in western Nigeria. Archives of Microbiology. 2009;**191**(8):639-648