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Comparative herbal and conventional antimicrobial susceptibility patterns of methicillin-resistant (MR) and methicillin-susceptible (MS) staphylococci of clinical and environmental origin

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Abbreviations: *CLSI, Clinical Laboratory Standards Institute; MHA, Mueller Hinton agar; MR, cefoxitin-resistant; UTI, urinary tract infection; MDR, multi-drug-resistant; MRSA, methicillin-resistant S. aureus; MSSA, methicillin-susceptible S. aureus, MRS, methicillin-resistant staphylococci; MSS, methicillin-susceptible staphylococci; CNS, coagulase-negative staphylococci; VRSA, vancomycin-resistant S. aureus; VSSA, vancomycin-susceptible S. aureus; VRS, vancomycin-resistant staphylococci; VSS, vancomycin-susceptible staphylococci; MHDR, multiple herbal antimicrobial; MDR, multiple drug resistance; OR, Odds ratio; CI_{99}, confidence interval at 99%; AST, antibiotic susceptibility testing; BHICV, Brain–Heart Infusion–Casein–Vancomycin; BHI, Brain–Heart Infusion; CDC, Centre for Disease Control; MIC, minimum inhibitory concentration; µg, microgram; AC, amoxicillin +clavulanic acid; Amx, amoxicillin; A, ampicillin; Az, azithromycin; Cp, cefepime; CTX, Cefotaxime; Cx, cefoxitin; C, chloramphenicol; Cf, ciprofloxacin; Cd, clindamycin; Cot, cotrimoxazole; Do, doxycycline; E, erythromycin; g, Gentamicin; I, imipenem; Ln, lincomycin; Lz, linezolid; Mp. meropenem; Mi, minocycline; Nf, nitrofurantoin; P, penicillin; Pi, piperacillin; PiT, piperacillin + tazobactam; T, tetracycline; Tig, tigecycline; V, vancomycin; AO, ajowan oil; BLO, betel leaf oil; Car, carvacrol; CNH, cinnamaldehyde; CO, cinnamon oil; GO, guggul oil; HBO, holy basil oil; LGO, lemongrass oil; SWO, sandalwood oil; TO, thyme oil.*

ABSTRACT

*Staphylococci are the leading cause of bacteraemia (septicemia), infective endocarditis (infection in the heart), osteoarticular infections (joints' infection), skin and soft tissue infections, pleuropulmonary (lung and respiratory tract infections), nosocomial (hospital borne) infections in human and animals. Specifically, vancomycin, methicillin and multi-drug resistant (MDR) staphylococci lead to millions of deaths every year. However, little is understood about methicillin resistance and MDR in staphylococci strains of coagulase-negative or non-S. aureus staphylococci. The present study targeted the void concerning the relation of methicillin resistance with multiple antimicrobial resistances among different species of staphylococci.**I**n the study staphylococci from clinical (607) and non-clinical (267) sources isolated at ICAR-Indian Veterinary Research Institute, Izatnagar, India, were tested for their methicillin resistance using an alternative test (cefoxitin resistance) and susceptibility of the strains to 39 antimicrobials was conducted as per standard CLSI protocol. The data was analyzed to determine the significance of species of the host of origin and species of staphylococci strains and resistance patterns in Microsoft Excel.Staphylococci strains included in the study belonged to 26 species;S. epidermidis was the most commonly identified species from clinical samples followed by S. aureus, S. intermedius,and S. haemolyticus. From non-clinical samples, too S. epidermidis was the most commonly identified Staphylococcus followed by S. haemolyticus, S. aureus and S. intermedius strains. In the study, S. saprophyticus and S. warneri strains were isolated only from non-clinical and clinical samples, respectively. Occurrence of cefoxitin resistance (or methicillin resistance), and multi-drug resistance (MDR) were slightly higher in clinical staphylococci (62.44%, 55.52%) than in non-clinical staphylococci (62.17%, 51.31%) but more concerning was multiple-herbal drug-resistance (MHDR) detected in 60.30% of non-clinical and 42.17% of the clinical strains. The most effective antibiotic against staphylococci was minocycline followed by imipenem, tigecycline, chloramphenicol, meropenem, piperacillin + tazobactam, nitrofurantoin, gentamicin, doxycycline, clindamycin, amoxicillin + clavulanic acid, linezolid, piperacillin, tetracycline and azithromycin, other 11 recommended antibiotics for*

staphylococcal infections failed to inhibit >65% of staphylococci. Of the 11 herbal antimicrobials tested, five herbal antimicrobials inhibiting ≥80% of staphylococci-causing infections were carvacrol, thyme oil, ajowan oil, cinnamaldehyde, and cinnamon oil revealing their potential as topical antimicrobials to treat skin infections by staphylococci. The present study revealed that methicillin resistance was rampant among both clinical and non-clinical staphylococci and not limited to S. aureus only but detected among all Staphylococcus species strains except S. capraestrains. However, the occurrence of MR varied among strains of different species. Vancomycin and linezolid, the recommended drugs for treating infections with MRSA had no significant difference in their efficacy concerning MR; however, MDR was significantly more common in MR than MS staphylococci. The study suggested the need to review the panel of antibiotics recommended for the treatment of staphylococcal infections in humans and animals.

Keywords: *Carvacrol, Thyme oil, Ajowan oil, Cinnamaldehyde, Cnnamon oil, MRSA, MDR, Minocycline, Tigecycline*

1.0 Introduction

Antimicrobial resistance (AMR) in environmental bacteria is ancient; resistance in human and animal pathogens emerged as a major problem after the rampant clinical use of antibiotics [10, 21, 23, 28, 36, 44]. Staphylococci are one of the most common groups of bacteria affecting all types of animals and humans and are the leading cause of bacteremia, infective endocarditis, osteoarticular infections, skin and soft tissue, pleuropulmonary, and nosocomial infections [24, 45]. The major classes of AMR staphylococci are vancomycin-resistant (VRS) and methicillin-resistant (MRS) strains [19, 23]. Methicillin being a bit less stable is often not used directly to detect MRS strains and several indirect assays are used to determine MRS [30]. Commonly conventional CLSI methods are preferably used (CLSI) to detect MRS because molecular methods like detection of *mec*A (responsible for methicillin resistance (MR) gene may not be present in some of the strains having MR [9, 13]. The most commonly recommended methods to detect MRS are screening by cefoxitin disc diffusion method or oxacillin broth microdilution method and isolates are considered as MRS if they are found resistant to any of the two antibiotics irrespective of the presence of *mec*A gene [9, 13]. Often, vancomycin is considered the drug of choice for the treatment of infections caused by MRS [18].

For treatment of staphylococcal infections, CLSI recommended [9] antimicrobial susceptibility testing of staphylococci against four groups of antimicrobials; group A (primary group) consists of azithromycin, erythromycin, clarithromycin, clindamycin, oxacillin, cefoxitin, penicillin G, and trimethoprim-sulphamethoxazole (co-trimoxazole); group B (used selectively) includes doxycycline, minocycline, tetracycline, linezolid and vancomycin; group C (used selectively with supporting evidence) has chloramphenicol, ciprofloxacin, moxifloxacin, and gentamicin; and group U (used as complementary antibiotics for treatment of urinary tract infection), has two antimicrobials namely nitrofurantoin and cotrimoxazole.

There are lot many reports on the occurrence of MRS strains in humans and animals [23, 45], but only a few on the comparative susceptibility of MRS strains to different antimicrobials to aid the selection of conventional antimicrobials and the possibility of herbal antimicrobials as an alternate [2, 37, 39, 41, 42]or in combination with antibiotics [5, 6] for therapeutic use. The present study was undertaken to compare the antimicrobial susceptibility of cefoxitin-resistant (MR) and cefoxitin-susceptible (MS) staphylococci isolated from clinical and non-clinical samples of different origin. In the study, 11 herbal antimicrobials known for their in-vitro efficacy against many bacteria [37, 39-42] including ajowan (*Tachyspermumammi*) oil, betel (*Piper betel*) leaf oil, carvacrol, holy basil (*Ocimum sanctum*) oil, citral, cinnamon (*Cinnamomum verum*) oil, cinnamledehyde, guggul (*Commiphorawightii*) oil, lemongrass (*Cymbopogon citratus*) oil, sandalwood (*Santalum album*) oil and thyme (*Thymus vulgaris*) oil were tested for their efficacy on staphylococci. Staphylococci were also tested for their antimicrobial susceptibility to 26 antimicrobials recommended by CLSI [9] and some of the newer antibiotics including amoxicillin, amoxicillin + clavulanic acid, ampicillin, azithromycin, cefepime, cefotaxime, cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, cotrimoxazole, doxycycline, erythromycin, gentamicin, imipenem, lincomycin, linezolid, meropenem, minocycline, nitrofurantoin, penicillin G, piperacillin, piperacillin + tazobactam, tetracycline, tigecycline, and vancomycin.

2.0 Materials and Methods

2.1 Bacterial strains: A total of 874 *Staphylococcus* species strains isolated from clinical (607) and non-clinical (267) samples and known for their susceptibility to cefoxitin were revived from glycerol stocks available in the repository of the Clinical Epidemiology Laboratory, Division of Epidemiology, ICAR-Indian Veterinary Research Institute, Izatnagar-243 122, India. The isolates included in the study were isolated between January 2015 and December 2023 from referred clinical samples of different hosts with different types of illnesses. After revival, all isolates were checked for purity and identity using biochemical characterization [7, 35].

2.2 Antimicrobial susceptibility testing

All the strains were tested using BD BBL Sensi-Discs (BD, Sparks, USA) for antimicrobial susceptibility testing on Mueller Hinton agar (MHA, BD BBL, USA) using amoxicillin (30 µg), amoxicillin + clavulanic acid (30 +10 µg), ampicillin (10 µg), azithromycin (15 µg), cefepime (30 µg), cefotaxime (10 µg), cefoxitin (10 µg), chloramphenicol (25 µg), ciprofloxacin (10 µg), clindamycin (10 µg), cotrimoxazole (25 µg), doxycycline (30 µg), erythromycin (15 µg), gentamicin (30 µg), imipenem (10 µg), lincomycin (10 µg), linezolid (30 µg), meropenem (10 µg), minocycline (30 µg), nitrofurantoin (300 µg), penicillin G (10 u), piperacillin (100 µg), piperacillin + tazobactam (100+10 µg), tetracycline (30 µg), and tigecycline(30 µg) discs on MHA. The results of antimicrobial susceptibility were interpreted according to CLSI guidelines [9, 13]. To determine vancomycin susceptibility, the vancomycin agar screen test was used [8].

2.3 Herbal antimicrobial susceptibility testing

All staphylococci strains were tested using herbal diffusion assay [39, 40], Herbal antimicrobial discs were loaded with 1 mg of ≥99% pure active herbal compounds. The herbal discs were made with carvacrol, citral, cinnamaldehyde, cinnamon (*Cinnamomum verum*) oil, lemongrass (*Cymbopogon citratus*) oil procured from Sigma Aldrich, USA; ajowan (*Tachyspermumammi*) oil, betel (*Piper betel*) leaf oil, holy basil (*Ocimum sanctum*) oil, sandalwood (S*antalum album*) oil, and thyme (*Thymus vulgaris*) oil procured from Nagaland Fragrance Pvt. Ltd (Dimapur, India) and guggul (*Commiphorawightii*) oil

In non-clinical samples including healthy humans, animals, foods, air, water and surfaces etc. the most common *Staphylococcus* species identified were similar as in clinical samples but with varied frequencies viz, *S. epidermidis* (16.85%), *S. haemolyticus* (13.11%), *S. aureus* (12.73%), *S. arlettae* (8.87%), *S. intermedius* (16.85%), *S. capitis* (5.24%), *S. chromogenes*(4.125%), *S. dolphin* (3.75%), *S. xylosus*(3.75%), *S. hominis* (3.37%), *S. caprae*(2.62%), *S. saprophyticus* (2.62%), *S. felis*(225%), *S. scleiferi*(1.87%), *S. carnosus* (1.87%), *S. hyicus*(1.50%), *S. lugdunensis S. hyicus*(1.50%), *S. auricularis S. hyicus*(1.50%), *S. gallinarum*(1.50%), *S. cohnii*(1.12%), *S. equorum* (1.12%), *S. saccharoltyicus*(1.12%), *S. caseolyticus*(0.75%), *S. kloosi* (0.37%), *S. simulans*(0.37%), and *S. warneri* (0.00%).

Irrespective of the source of isolation, a significantly (p, ≤0.02) higher proportion of *S. aureus* isolates were MRS (cefoxitin resistant) type than *S. caprae* isolates. However, *S. aureus*causing infections were less often MRS type than *S. epidermidis* (p, 0.01). Non-clinical *S. aureus*strains were significantly less often resistant to cefoxitin than *S. carnosus* (p, 0.05) but more often (p, 0.03) than non-clinical *S. chromogenese* strains.

Staphylococcus arlettae and *S. carnosus* isolated from clinical samples were less often (p, ≤0.2) resistant to cefoxitin than those isolated from non-clinical samples. However, *S. chromogenese* from clinical samples were significantly (p, 0.2) more often resistant than non-clinical isolates.

From clinical samples, *S. aureus* isolates more commonly had MDR than strains of *S. caprae* (p, 0.01) and epidermidis (p, 0.03). From non-clinical samples, *S. aureus* isolates had MDR more often (p, ≤0.01) than strains of *S. cparae* and *S. felis*. Strains of *S. hyicus* (p, 0.03) and *S. xylosus* (p, <0.01) from clinical origin more often had MDR than those from non-clinical samples. The MDR was the least common among clinical isolates from deer, cattle, and horses and the most common among isolates from sick dogs, humans and poultry birds. The MDR strains were detected in significantly lower proportions in clinical samples of deer than those from clinical samples of buffaloes, cats, cattle, dogs, elephants, goats, horses, humans, poultry birds (p, ≤0.01), experimental animals (p, 0.04) and big cats (p, <0.05). Next to deer, a lesser number of clinical strains of cattle origin had MDR than strains isolated from clinical samples of cats (p, 0.04), poultry birds (p, 0.03), dogs, and humans (p, <0.01). Isolates from horses were also less often MDR type than strains from sick humans (p, 0.01).

Among non-clinical sources, human hands were the most common sources of MDR strains followed by foods and healthy animals. Staphylococci from apparently healthy animals were less often MDR type than those from environmental (air, water, and inanimate surfaces) sources (p, 0.01), fingertips of humans (p, <0.01), and milk and foods (p, 0.03). MDR was the least common among cefoxitin-susceptible staphylococci of non-clinical samples (p, ≤0.02), followed by cefoxitin-susceptible staphylococci from clinical samples, cefoxitin-resistant staphylococci from non-clinical and clinical samples. Non-clinical cefoxitin-resistant isolates of staphylococci more often had MDR than cefoxitin-susceptible staphylococci of clinical (p, 0.03) and non-clinical (p, <0.01) origin.

The most effective antimicrobials on staphylococci from UTI cases were tigecycline, minocycline, piperacillin + tazobactam, chloramphenicol, imipenem and nitrofurantoin inhibiting >86% of the staphylococci isolated from the urine of UTI cases, and cotrimoxazole failed to inhibit about 54% of the UTI isolates.

A total of 13.85% of staphylococci from 69 UTI infections were resistant to nitrofurantoin while 18.4% of 538 staphylococci associated with other infections were resistant to nitrofurantoin but they were more often (p, 0.01) susceptible to cotrimoxazole (62.45%) than staphylococci causing infections of the urinary tract (46.15%). Other antibiotics failed to inhibit a substantial number of UTI strains of staphylococci, viz., gentamicin (25.37%), vancomycin (30.61%), and ciprofloxacin (52.46%), while cefoxitin and penicillin G failed to inhibit 65.22% and 91.67% of staphylococci isolated from UTI samples.

Non-clinical isolates of staphylococci were more often resistant than clinical isolates to holy basil oil (HBO), cinnamaldehyde, lemongrass oil (LGO), thyme oil (TO), citral, cinnamon oil (CO), sandalwood oil (SWO), betel leaf oil (BLO), guggul oil (GO), imipenem, Amoxycillin, amoxicillin + clavulanic acid (amoxiclav), vancomycin, piperacillin, piperacillin-tazobactam, but it was reverse concerning clindamycin, tetracycline, doxycycline, and co-trimoxazole.

Non-clinical cefoxitin-resistant isolates of staphylococci (MRS) were more often resistant than clinical MRS isolates to HBO, cinnamaldehyde, LGO, TO, citral, CO, SWO, GO, BLO, imipenem, amoxycillin, amoxyclav, vancomycin, piperacillin, piperacillin-tazobactam, but it was reverse concerning doxycycline and cotrimoxazole.

Non-clinical cefoxitin-susceptible isolates of staphylococci (MSS) were more often resistant than clinical MSS isolates to citral, and piperacillin-tazobactam, but it was reversed for tetracycline, gentamicin, erythromycin, clindamycin and cefotaxime.

Staphylococci from healthy animals were more often susceptible than those from the hands of healthy humans to HBO, cinnamaldehyde, carvacrol, TO, SWO, penicillin, nitrofurantoin, chloramphenicol, azithromycin, erythromycin, clindamycin, amoxicillin, amoxyclav, cefotaxime, and piperacillin. Staphylococci from healthy animals were also more often susceptible than those from milk and other foods to citral, guggul oil, penicillin, ciprofloxacin, azithromycin, and meropenem, but more resistant to linezolid; more often susceptible than those from environmental samples to cefoxitin, holy basil oil, cinnamaldehyde, TO, SWO, BLO, penicillin, tetracycline, nitrofurantoin, azithromycin, erythromycin, meropenem, imipenem, cefotaxime, cefepime, and piperacillin, but more resistant to ciprofloxacin. Staphylococci from healthy human hand swabs were more often susceptible than those from environmental samples to HBO, CO, BLO, GO, nitrofurantoin, meropenem, imipenem, and cefepime, but more resistant to carvacrol, ciprofloxacin, linezolid and amoxiclav.

Staphylococci from healthy human hand swabs were more often resistant than those from milk and food samples to holy basil oil, cinnamaldehyde, lemongrass oil, linezolid, amoxiclav, and cefotaxime, but more susceptible to citral, sandalwood oil, and guggul oil.

Staphylococci from milk and foods were more often susceptible than those from environmental samples to holy basil oil, cinnamaldehyde, lemongrass oil, thyme oil, sandalwood oil, betel leaf oil, nitrofurantoin, linezolid, and imipenem, but more resistant to citral and ciprofloxacin.

The AMR in staphylococci isolated from clinical samples of various hosts varied significantly from each other (Tab. 2). Though minocycline was the most effective antimicrobial inhibiting 97.74% of the staphylococci and penicillin G being the least effective among 39 of the antimicrobial tested, there was little variation in top 10 most effective antimicrobials (Tab. 3) on

staphylococci isolated from clinical samples of 14 groups of hosts and the distribution was almost normal falling under a bell-shaped curve (Fig. 1), the most effective and the least effective antimicrobials had less discrimination power among different staphylococci and most of the variability was evident for susceptibility to antimicrobial effective on 20-60% of the strains (Fig. 1). On human origin staphylococci tigecycline was the most effective antibiotic while on animal origin strains minocycline was the best followed by imipenem and tigecycline, but the difference was not statistically important. However, for herbal antimicrobials human and animal-origin staphylococci had almost similar susceptibility patterns (Tab. 4). There were 22 (six of herbal origin and 16 conventional antimicrobials) of the 39 antimicrobials among the most effective antimicrobials on staphylococci isolated from clinical samples from 14 different host species groups (Tab. 3). Tigecycline appeared in top 10 lists of antimicrobial for staphylococci infecting all 14 host groups, followed by carvacrol (13), imipenem (13), thyme oil (12), minocycline (12), piperacillin + tazobactam (12), ajowan oil (10), chloramphenicol (9), meropenem (8), clindamycin (6), gentamicin (5), cinnamaldehyde (4), cinnamon oil (4), nitrofurantoin (4), linezolid (3), azithromycin (3), doxycycline (2), ampicillin (2), amoxicillin (1), citral (1), vancomycin (1) and cotrimoxazole (1).

4.0 Discussion

Staphylococci are the leading cause of infections in humans and animals [24, 45] and drug resistance among staphylococci made them one of the top killer infectious agents, that too only MRSA [24, 27, 45]. The majority of the human-origin strains in study 41 (54.93%) were associated with urinary tract infections (UTIs) followed by skin infections, respiratory tract infections, otorrhoea, bacteraemia, intestinal abscesses, and metritis cases. Similar types of ailments associated with staphylococcal infection in humans have been reported earlier from different parts of the world (11, 24, 45]. Though staphylococci are known to cause many cases of UTI in humans [17] such a high proportion of staphylococci associated with UTIs in the present study may be attributed to the fact that the majority of human samples submitted to the laboratory are UTI-related. This is likely because other infections in the Bareilly region are often treated without conducting antimicrobial susceptibility testing. In the present study, of the 41 strains from human UTI cases only one was *S. aureus* while others were all coagulase-negative staphylococci (CNS). In earlier studies on staphylococcal UTIs, *S. saprophyticus* and other CNS have been considered more important than *S. aureus*[3, 11, 17] but in the present study, none of the *S. saprophyticus* isolates was associated with clinical-samples. Although nitrofurantoin and cotrimoxazole are the most recommended antimicrobials for UTI infections with staphylococci [9, 13], in the present study the most effective antimicrobials on staphylococci from UTI cases were tigecycline, minocycline, piperacillin + tazobactam, chloramphenicol, imipenem and nitrofurantoin inhibiting >86% of the staphylococci isolated from the urine of UTI cases, while cotrimoxazole failed to inhibit 53.85% of staphylococci causing UTIs. Other antibiotics that failed to inhibit a substantial number of UTI strains of staphylococci were gentamicin (25.37%), vancomycin (30.61%), and ciprofloxacin (52.46%), while cefoxitin and penicillin G failed to inhibit 65.22% and 91.67% of staphylococci isolated from UTI samples, respectively. In a recent study in Benin on staphylococci from UTI cases [4] gentamicin inhibited 73.1% of the strains similar to our observations (74.6%) but vancomycin (42.3%) and cotrimoxazole (96.2%) resistance were reported at much higher levels [4] than observed in the current study, it might be a regional difference leadingto the prevalence of different types of staphylococci varying in their susceptibility.

In the present study, from veterinary clinical samples staphylococci were most commonly isolated from skin infections, bacteraemia/ septicemia, mastitis, metritis, otorrhoea, conjunctivitis, urinary tract infections, respiratory tract infections, aborted foeti, intestinal abscesses, joint infections, naval ill and gum abscess. Staphylococci (*S. aureus* and CNS) are known to cause similar type of infections earlier in animals [26, 32, 34].

In clinical samples, the most common *Staphylococcus* was *S. epidermidis* (in 22.24% of samples), followed by *S. aureus* (14.99%), *S. intermedius* (14.17%), *S. haemolyticus*(13.51%). The observations are not in concurrence with earlier observations in Oregon [28] reporting *S. aureus* in 12%, and *S. intermedius* in 11% of the samples. However, in Oregon[34], *S. epidermidis* was not the most common staphylococci instead it was *S. pseudintermedius* (28%). The discrepancy might be because *S. pseudintermedius* may not be common in the Bareilly region as it was not detected in any of the clinical or non-clinical samples and *S. epidermidis* might have occupied that niche in Bareilly. None of the non-clinical samples had *S. warneri*similar to the Oregon study in the USA [34] where *S. epidermidis* and *S. hominis* strains were detected in a sizeable number of non-clinical samples. The occurrence of similar types of staphylococci in clinical and non-clinical samples in the present study further emphasizes that staphylococci commensally inhabit opportunistic pathogens distributed in healthy as well as sick hosts [46]. Staphylococci are known to inhabit the noses of up to 40% of apparently healthy humans from there they may spread anywhere to find an opportunity to cause infection [29]. Staphylococci isolated from clinical and non-clinical samples has methicillin resistance (cefoxitin resistance) in 62.44% and 63.67% of strains, respectively indicating the equitable distribution of MRS strains in both types of samples again supporting opportunistic pathogen nature of staphylococci. The results are in concurrence with global observation reporting MR is more than half of the staphylococci [16]. However, there was a significant difference in methicillin and other antimicrobial susceptibility of staphylococci of different species viz., irrespective of source of isolation, significantly ($p, \leq 0.02$) higher proportion of *S. aureus* isolates was MRS type than *S. caprae* isolates. Similar observations are made earlier also indicating differences in the MRS status of staphylococci depending on species [37, 43].

The antimicrobial resistance in staphylococci isolated from clinical samples of various hosts varied significantly from each other in concurrence with earlier reports [43].In the present study, MDR was detected in 55.52% of clinical isolates and 51.31 non-clinical isolates but was more common among staphylococci isolates from sick dogs, humans and poultry birds (64%), it might be due to exposure to bacteria to wider spectrum of antibiotics [14, 36] than used in cattle and buffaloes (44%). Similarly, MRS was also more common in staphylococci from sick dogs, humans, and poultry birds (63.7%) than in staphylococci infecting cattle and buffaloes (59.7%), probably due to the same reason as for MDR. In the present study, linezolid (OR, 2.44, CI_{99}9 1.39-4.25) was significantly less effective against MRS strains than on MSS strains but no significant difference was observed concerning vancomycin susceptibility.

Further, MDR strains were more often resistant to linezolid (OR, 2.69, CI_{99} 1.57-4.62) and vancomycin (OR, 1.99, CI_{99} 1.32-3.01) than non-MDR strains of staphylococci and jeopardized the claims made in earlier studies reporting that vancomycin and linezolid should be drugs of choice for treatment of MRS and MDR staphylococcal infections [14, 15].

The MDR was the least common among cefoxitin-susceptible staphylococci (MSS) of non-clinical samples (p, ≤0.02), followed by cefoxitin-susceptible staphylococci from clinical samples, and cefoxitin-resistant staphylococci from non-clinical and clinical samples. Further, methicillin-resistant CNS strains more often (OR 1.71, CI_{99} 1.14-2.55) had multiple-drug-resistance (MDR) than methicillin-susceptible CNS strains, but no such difference was observed concerning MRSA and non-MRSA strains irrespective of their origin. However, in earlier studies [1] in Nepal (a nearby country) and Pakistan [27, 33]MDR is reported markedly higher among MRSA than MSSA strains. The difference might be the spectrum of sources of staphylococci [43], only human isolate included in the Nepal study and staphylococci were from more diverse sources in the present study. However, the resistance of MRSA strains to gentamicin (25%) in this study is in concurrence with observations in the earlier study [1] reporting gentamicin resistance in 27.4% of their MRSA strains but in contrast to chloramphenicol resistance in 8.67% MRSA, they [1] reported it in 17.9% strains.

The MDR was less common in clinical isolates from herbivores (deer, cattle, and horses) and was the most common among isolates from sick dogs, humans, and poultry birds. Though few studies are available comparing MDR in staphylococci of different origins[43], there seems to be scanty information on the comparison of a wide range of clinical and non-clinical staphylococcal isolates as done in the present study. Further, MDR strains were less often detected in horses and other herbivore types than those from sick humans (p, 0.01). Further, among non-clinical sources, human hands were the most common sources of MDR strains followed by foods and healthy animals. Food probably being contaminated while handled by humans might have got MDR staphylococci.

Though minocycline is one of the group B recommended antibiotics [7, 27] for staphylococci gentamicin is often reported as one the most effective antimicrobials on MRSAs [1, 14, 43], in our study minocycline was the most effective antimicrobial inhibiting 97.74% of the staphylococci and penicillin G being the least effective of the 39 antimicrobials tested. Though there was little variation in the top 10 most effective antimicrobials on staphylococci from 14 different groups of hosts, there were six herbal and 16 conventional antimicrobials that appeared as the most effective antimicrobials on staphylococci isolated from clinical samples. Among the topmost effective conventional antimicrobials, tigecycline appeared in top 10 lists of antimicrobials for staphylococci infecting all 14 host groups, followed by imipenem (13), minocycline (12), piperacillin + tazobactam (12), chloramphenicol (9), meropenem (8), clindamycin (6), gentamicin (5), nitrofurantoin (4), linezolid (3), azithromycin (3), doxycycline (2), ampicillin (2), amoxicillin (1), vancomycin (1) and cotrimoxazole (1). In most of the earlier studies, similar types of antibiotics have been found effective against staphylococcal infections with some variations [1, 12, 14, 25, 31, 33, 43, 47].

Though clindamycin was referred as the preferred outpatient antibiotic therapy for staphylococcal infections [25, 47], it was not among the top 10 most effective antimicrobials on staphylococci isolated from humans, cattle, deer, and dogs, horses, poultry birds, goats and sheep in the present study indicating that after a decade of clindamycin exposure scenario has changed a lot.Though many different reasons have been given for emergence of AMR and MDR in bacteria including staphylococci, a prior exposure of bacteria in host or environment is considered as the most important driver for emergence and spread of AMR [22, 36, 44].Four of the herbal antimicrobials (carvacrol, thyme oil, ajowan oil, and cinnamaldehyde) inhibited the majority of the staphylococci infecting humans and animals. Similar observations are reported earlier on a wide variety of pathogens including staphylococci revealing that carvacrol (active ingredient of ajowan oil, thyme oil, and oregano oil) and cinnamaldehyde (active ingredient in cinnamon oil) are the best herbal compounds possessing the potential for development therapeutic herbal antimicrobial [6]. Herbal antimicrobials have been seen as important alternatives and supportive antimicrobial therapeutic agents [2, 5, 37], a lot of research is needed to utilize herbal agents because of their inherent toxic potential and problems of suitable delivery vehicles [20, 38].

5.0 Study Strengths and Limitations

The major strength of the study is exhaustiveness of the study including staphylococci of 26 species from 14 different groups of hosts and from different ailments. Further, statistical analysis to find out the impact of methicillin resistance on a number of other antibiotic resistances and MDRhas rarely been reported earlier. The study lucidly explains what antibiotics may be more useful for different types of staphylococci and in different hosts suffering from different disease conditions of staphylococcal infections.The major limitation of the present study was the inclusion of staphylococci isolated from samples of referred cases often having previous antibiotic treatment history, no quantitative determination of antimicrobial resistance using minimum inhibitory concentration (MIC) assays and no genotypic method or molecular deterministic method was used for confirming the identity of the strains of different staphylococcal species.

6.0 Conclusions

The study concluded that methicillin resistance is not only rampant in *S. aureus* but present among all different species of *Staphylococcus* genus but with variations in prevalence. The MRSA strains did not differ significantly from MSSA in their susceptibility to vancomycin and linezolid, the recommended drugs for treating infections with MRSA. Multi-drug resistance was significantly more in cefoxitin-resistant staphylococci (MRS) than in cefoxitin susceptible strains. The study indicated the need for a review of antibiotic recommendations for therapeutic use against infections with MRS and MDR strains. Further, study revealed the potential of herbal antimicrobial for the development of potential therapy for staphylococcal infections.

Tab. 1. Cefoxitin-resistance (MRSA), multiple herbal antimicrobial drug resistance (MHDR) and multiple antimicrobial drug resistance (MDR) in staphylococci of different species isolated from clinical and non-clinical samples

Staphyloco-ccus species	Clinical isolates				Non-clinical isolates			
	N	MRSA	MHDR	MDR	N	MRSA	MHDR	MDR
S. arlettae	11	6	7	7	21	19	20	13
S. aureus	91	52	44	57	34	19	16	17
S. auricularis	7	5	1	5	4	1	3	3
S. capitis	43	28	20	18	14	9	10	8
S. caprae	4	0	1	0	7	0	0	0
S. carnosus	1	0	0	1	5	5	3	5
S. caseolyticus	8	4	3	4	2	0	0	0
S. chromogenes	18	11	10	7	11	2	6	5
S. cohnii	16	7	4	10	3	2	3	2
S. delphini	14	8	7	9	10	8	9	7
S. epidermidis	135	99	59	65	45	30	19	25
S. equorum	2	2	1	0	3	0	3	2
S. felis	7	5	2	3	6	4	6	0
S. gallinarum	4	3	2	1	4	3	2	1
S. haemolyticus	82	45	40	41	35	25	24	18
S. hominis	11	8	7	10	9	6	7	5
S. hyicus	24	12	6	19	4	4	1	1
S. intermedius	86	53	29	50	19	11	12	10
S. kloosi	5	4	1	3	1	1	1	1
S. lugdunensis	9	3	4	5	4	2	1	2
S. sacchroltyicus	3	3	0	2	3	0	0	2
S. saprophyticus	0	0	0	0	7	4	4	4
S. schleiferi	11	9	5	8	5	3	3	3
S. simulans	1	1	0	1	1	1	1	1
S. warneri	4	3	0	3	0	0	0	0
S. xylosus	10	8	3	8	10	7	7	2
Total	607	379 (62.44%)	256 (42. 17%)	337 (55.52%)	267	166 (62.17%)	161 (60.30%)	137 (51.31%)

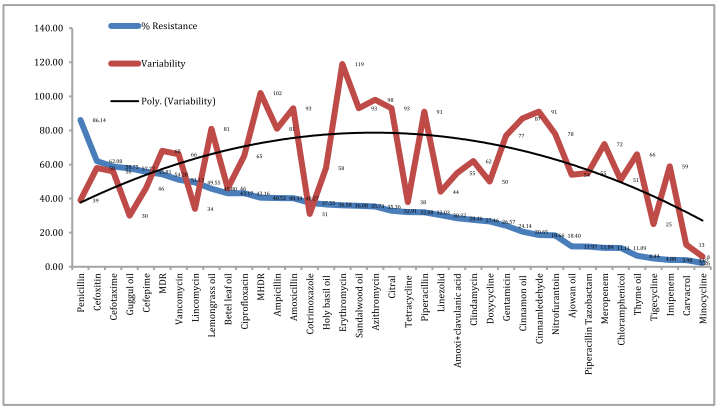

Fig.1. Antimicrobial resistance (AMR) variability (significant p, ≤ 0.05) among staphylococci: Resistance to different antimicrobials and variability among different staphylococci with respect to resistance to the specific antimicrobial (variability is less for both highly effective and less effective antimicrobials).

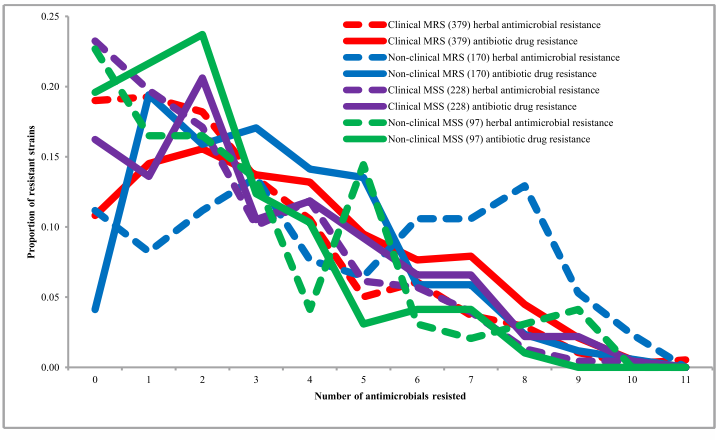

Fig. 2. Distribution of multiple conventional antimicrobial and herbal antimicrobial resistances in clinical and non-clinical in relation to their methicillin resistance.

Table. 2. Comparative antimicrobial resistance in staphylococci isolated from clinical samples of different host species

Staphylococci from clinical samples of	Significantly more resistant (MR) or significantly more susceptible to different antibiotics with respect to clinical sample source of staphylococci isolated																							
	Big Cats		Buffaloes		Cattle		Deer		Dogs		Elephants		Horse		Humans		Pig		Poultry		Sheep and Goats		Wild birds	
	MR	MS	MR	MS	MR	MS	MR	MS	MR	MS	MR	MS	MR	MS	MR	MS	MR	MS	MR	MS	MR	MS	MR	MS
Big cats	0	0	AO, CNH, TO, L	0	AO, CNH, CO, SWO, T, Mi, Mp, L	0	AO, CNH, LGO, citral, CO, SWO, T, G, Az, E, Mp	0	AO, HBO, CNH, CO, SWO, Mi, L	0	CO, T, Mi, Cot, Cf, Amx	0	AO, HBO, CNH, Citral, Mp	0	CNH, SWO, Mi	0	0	0	BLO, LZ, A, Mp, Pi	0	LGO, Citral, SWO, L	0	AO, CO, SWO, GO, L	0
Buffaloes	0	AO, CNH, TO, L	0	0	HBO, BLO, Do, Nf, A, Mp, I,	0	LGO, SWO, BLO, Cot, Cf, Az, E, Mp	Cd	HBO, LGO, SWO, BLO, Do, Mp, I	V	T, Do, A, Mp, I, Amx, Pi, PiT	AO, CNH, NF, L	HBO, Citral, BLO, C, Mp, I	0	BLO, Do, Mp	0	BLO, Az	To	BLO, A, Mp, Pi, PiT	To, Cd	LGO, SWO, BLO, Do	To	MRS, BLO	0
Cattle	0	AO, CNH, CO, SWO, T, Mi, Mp, L	0	HBO, BLO, Do, Nf, A, Mp, I,	0	0	CNH, G, Az	Cd	AO,CNH, BLO	T, Cot, Cf, Az, E, A	Cf, Amx	AO, HBO, CNH, NF, A, L	AO, Citral, C	T, Do	Tig	LGO, CO, GO, Cot, Cf, Az, E, A	0	LGO	BLO, P, Lz, A, Pi	Co, T, Nf, Cf, Az, E, Cd	0	Carvacrol, Nf, E, Mp	HBO	`A
Deer	Cd	AO, CNH, LGO, citral, CO, SWO, T, G, Az, E, Mp	Cd	LGO, SWO, BLO, Cot, Cf, Az, E, Mp	Cd	CNH, G, Az	0	0	Cd	T, G, Cot, Cf, Az, E,	Cd, A, Amx	AO, HBO, CNH, LGO, G, NF, A, L	Cd	T, G, Cot, Az, E,	Cd	LGO, CO, GO, G, Cot, Cf, C, Az, E	Cd	LGO, Citral	A	CNH, LGO, Citral, CO, T, Nf, Cf, Az, E	Cd	CNH, Carvacrol, Cot, C, Az, E	Cd	G, Az
Dogs	0	AO, HBO, CNH, CO, SWO, Mi, L	V	HBO, LGO, SWO, BLO, Do, Mp, I	T, Cot, Cf, Az, E, A	AO ,CNH, BLO	T, G, Cot, Cf, Az, E,	Cd	0	0	P, Cot, Cf, Az, E, A, Amx	AO, HBO, CNH, LGO, Nf, L	Citral, Cot, C,	Go, Do	0	AO, HBO, LGO, CO, BLO, GO, C, Az, E	Az	LGO, Citral	P, Cot, A, Pi	HBO, LGO, Co, Nf, Cd	Citral	Carvacrol, C	MRS, BLO	0
Elephants	Nf, Mp	CO, T, Mi, Cot, Cf, Amx	AO, CNH, NF, L	T, Do, A, Mp, I, Amx, Pi, PiT	AO, HBO, CNH, NF, A, L	Cf, Amx	AO, HBO, CNH, LGO, G, NF, A, L	Cd, A, Amx	AO, HBO, CNH, LGO, Nf, L	P, Cot, Cf, Az, E, A, Amx	0	0	AO, HBO, CNH, Nf	T, Do, Cf, A, Amx	AO, HBO, CNH, Nf	Cot, Cf, Az, E, A, Amx, Pi	To, Citral, Cf, Amx	To, Citral, Cf, Amx	AO, P, Nf	To, CO, T, Cf, Az, E	AO, HBO, CNH, Carvacrol, LGO, Nf	T, Cot, Cf, C, E, A, Mp, Amx, Pi, PiT	AO, HBO, CNH, Nf	0
Horse	0	AO, HBO, CNH, Citral, Mp	0	HBO, Citral, BLO, C, Mp, I	T, Do	AO, Citral, C	T, G, Cot, Az, E,	Cd	Go, Do	Citral, Cot, C,	T, Do, Cf, A, Amx	AO, HBO, CNH, Nf	0	0	0	AO, HBO, LGO, Citral, Cot, Cf, C, Az, E, I	0	LGO, Citral	P, Lz, A, Pi	Carvacrol, Citral, Nf, E	Do	Carvacrol, C, Amx	Go	C, I
Humans	0	CNH, SWO, Mi	0	BLO, Do, Mp	LGO, CO, GO, Cot, Cf, Az, E, A	Tig	LGO, CO, GO, G, Cot, Cf, C, Az, E	Cd	AO, HBO, LGO, CO, BLO, GO, C, Az, E	0	Cot, Cf, Az, E, A, Amx, Pi	AO, HBO, CNH, Nf	AO, HBO, LGO, Citral, Cot, Cf, C, Az, E, I	0	0	0	Az, E	0	BLO, P, Cot, A, Pi	Nf	LGO, Citral, SWO, L	Carvacrol, Tig	CO, GO, Cf, Az	0
Pigs	0	0	To	BLO, Az	LGO	0	LGO, Citral	Cd	LGO, Citral	Az	To, Citral, Cf, Amx	To, Citral, Cf, Amx	LGO, Citral	0	0	Az, E	0	0	P, A, Pi	Az, E, Cd	LGO, Citral	Az, E	Citral	0

Poultry	0	BLO, LZ, A, Mp, Pi	To, Cd	BLO, A, Mp, Pi, PiT	Co, T, Nf, Cf, Az, E, Cd	BLO, P, Lz, A, Pi	CNH, LGO, Citral, CO, T, Nf, Cf, Az, E	A	HBO, LGO, Co, Nf, Cd	P, Cot, A, Pi	To, CO, T, Cf, Az, E	AO, P, Nf	Carvacrol, Citral, Nf, E	P, Lz, A, Pi	Nf	BLO, P, Cot, A, Pi	Az, E, Cd	P, A, Pi	0	0	LGO, Citral, Cd	P, Cot, C, A, Mp, Pi	A, Pi	CO, Az, Cd
Sheep and Goats	0	LGO, Citral, SWO, L	To	LGO, SWO, BLO, Do	Carvacrol, Nf, E, Mp	0	CNH, Carvacrol, Cot, C, Az, E	Cd	Carvacrol, C	Citral	T, Cot, Cf, C, E, A, Mp, Amx, Pi, PiT	AO, HBO, CNH, Carvacrol, LGO, Nf	Carvacrol, C, Amx	Do	Carvacrol, Tig	LGO, Citral, SWO, L	Az, E	LGO, Citral	P, Cot, C, A, Mp, Pi	LGO, Citral, Cd	0	0	0Ctx	0
Wild birds	0	AO, CO, SWO, GO, L	0	MRS, BLO	0	0	G, Az	Cd	0	MRS	0	AO, HBO, CNH, Nf	C, I	Go	0	CO, GO, Cf, Az	0	Citral	CO, Az, Cd	A, Pi	0	Ctx	0	0

AC, Amoxicillin +clavulanic acid; Amx, Amoxicillin; A, Ampicillin; Az, Azithromycin; Cp, Cefepime; CTX, Cefotaxime; Cx, Cefoxitin; C, Chloramphenicol; Cf, Ciprofloxacin; Cd, Clindamycin; Cot, Cotrimoxazole; Do, Doxycycline; E, Erythromycin; G, Gentamicin; I, Imipenem; Ln, Lincomycin; Lz, Linezolid; Mp. Meropenem; Mi, Minocycline; Nf, Nitrofurantoin; P, Penicillin; Pi, Piperacillin; PiT, Piperacillin + Tazobactam; T, Tetracycline; Tig, Tigecycline; V, Vancomycin.

AO, Ajowan oil; BLO, Betel leaf oil; Car, Carvacrol; CNH,Cinnamaldehyde; CO, Cinnamon oil; GO, Guggul oil; HBO, Holybasil oil; LGO, Lemongrass oil; SWO, Sandalwood oil; TO, Thyme oil.

Tab. 3. Most effective antimicrobials on Staphylococci causing infections in different animals

Buffaloes (32)	**Big cats (Leopards, lions, tigers) (14)**	**Cats (3)**	**Cattle (102)**	**Deer (17)**	**Dogs (242)**	**Elephants (22)**	**Exp. Animals (guinea pigs, rats) (3)**	**Horses (47)**	**Humans (75)**	**Pigs (7)**	**Poultry (19)**	**Sheep and goats (15)**	**Wild bids (Falcon, Golden finch, peacocks, pigeons) (9)**
TO (100)	Carvacrol (100)	TO (100)	Mi (100)	Mi (100)	Mi (96.85)	Carvacrol (100)	I (100)	I (100)	Tig (98.44)	Tig (100)	Tig (100)	I (93.33)	Carvacrol (100)
Mi (100)	PiT (100)	Tig (100)	I (97.94)	Carvacrol (100)	Carvacrol (96.09)	PiT (100)	G (100)	Carvacrol (100)	Mi (97.56)	Mi (100)	Mi (100)	Mi (92.86)	PiT (100)
Tig (94.74)	Cd (100)	Carvacrol (100)	Carvacrol (95.05)	PiT (100)	Tig (95.65)	Cd (100)	TO (100)	C (100)	Carvacrol (95.83)	Carvacrol (100)	I (100)	G (92.86)	Cd (100)
Carvacrol (93.55)	I (92.86)	AO (100)	Mp (94.68)	TO (100)	I (94.98)	I (100)	Tig (100)	Mi (100)	TO (94.12)	I (100)	Mp (100)	AO (86.67)	Tig (100)
AO (90.32)	C (92.86)	I (100)	Nf (91.75)	Tig (100)	TO (93.12)	Tig (100)	PiT (100)	Tig (97.44)	I (91.67)	Mp (100)	PiT (100)	TO (86.67)	Mi (100)
C (90)	Tig (91.67)	Nf (100)	PiT (91.03)	C (100)	AO (92.27)	TO (100)	CO (100)	AO (97.44)	Mp (86.57)	AO (100)	A (100)	Citral (86.67)	CO (100)
CNH (86.21)	TO (85.71)	PiT (66.67)	TO (90.43)	G (100)	C (89.09)	Mi (100)	Cd (100)	TO (94.29)	Nf (86.30)	C (100)	C (94.74)	Do (86.67)	AO (100)
Cd (85.71)	Mi (80)	Amx (66.67)	Tig (90.12)	Do (100)	Mp (87.56)	Mp (100)	Carvacrol (100)	Mp (91.30)	PiT (86.27)	Lz (100)	Lz (91.67)	Tig (84.62)	I (88.89)
I (83.87)	G (78.57)	CO (50)	C (88.24)	CNH (100)	CNH (87.50)	V (100)	C (100)	Nf (99.74)	CNH (83.58)	Cd (100)	Carvacrol (89.47)	PiT (80)	TO (88.89)
G (81.25)	A (72.73)	Mp (50)	AO (85.15)	Az (100)	PiT (86.63)	SWO (100)	AO (66.67)	PiT (86.45)	AO (83.33)	Az (100)	Cot (87.50)	CO (80)	Az (88.89)

AC, Amoxicillin +clavulanic acid; Amx, Amoxicillin; A, Ampicillin; Az, Azithromycin; Cp, Cefepime; CTX, Cefotaxime; Cx, Cefoxitin; C, Chloramphenicol; Cf, Ciprofloxacin; Cd, Clindamycin; Cot, Cotrimoxazole; Do, Doxycycline; E, Erythromycin; G, Gentamicin; I, Imipenem; Ln, Lincomycin; Lz, Linezolid; Mp. Meropenem; Mi, Minocycline; Nf, Nitrofurantoin; P, Penicillin; Pi, Piperacillin; PiT, Piperacillin + Tazobactam; T, Tetracycline; Tig, Tigecycline; V, Vancomycin.

AO, Ajowan oil; BLO, Betel leaf oil; Car, Carvacrol; CNH, Cinnamaldehyde; CO, Cinnamon oil; GO, Guggul oil; HBO, Holybasil oil; LGO, Lemongrass oil; SWO, Sandalwood oil; TO, Thyme oil.

Table 4. Comparative efficacy of different antimicrobials on staphylococci of human origin and animal origin

The most effective antimicrobial on staphylococci infecting (on >80% of the isolates)			
Humans	**% Resistant**	**Animals**	**% Resistant**
Tigecycline	1.56	Minocycline	2.69
Minocycline	2.44	Imipenem	4.40
Imipenem	8.33	Tigecycline	4.99
Meropenem	13.43	Chloramphenicol	9.85
Nitrofurantoin	13.70	Piperacillin Tazobactam	11.47
Piperacillin Tazobactam	13.73	Meropenem	11.56
		Nitrofurantoin	18.50
Herbal antimicrobials			
Carvacrol	4.17	Carvacrol	4.11
Thyme oil	5.88	Thyme oil	7.35
Cinnamaldehyde	16.42	Ajowan oil	11.72
Ajowan oil	16.67	Cinnamaldehyde	18.24
The least effective antimicrobial on staphylococci infecting (on <50% of the isolates)			
Humans	% Resistant	Animals	% Resistant
Penicillin	88.64	Penicillin	86.44
Azithromycin	60.27	Cefoxitin	62.97
Cefotaxime	60.00	Cefotaxime	60.24
Ciprofloxacin	59.70	Cefepime	56.49
Cefoxitin	58.67		
Lincomycin	57.45		
Erythromycin	55.41		
Ampicillin	54.93		
Cotrimoxazole	50.70		
The least effective herbal antimicrobial on staphylococci infecting			
Guggul oil	76.67	Guggul oil	68.25
Lemongrass oil	63.24	Lemongrass oil	58.73
Betel leaf oil	51.79	Betel leaf oil	55.56

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