

Research Article



Antimicrobial Susceptibility Profile of *Staphylococcus aureus* Isolates Recovered from Various Animal Species

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Abstract | *Staphylococcus aureus*, a normal flora of human and animals is recognized as an opportunistic pathogen that cause several infections. In present study, eighty *S. aureus* isolates were recovered from different animals such as sheep, goat, buffalo, camel, horse, cattle, dog and human. These isolates were exposed to in vitro antibiotic sensitivity test using eleven antimicrobial agents which included ampicillin (10µg), amikacin (30µg), chloramphenicol (30µg), sulfanilamide (30µg), erythromycin (15µg), gentamicin (10µg), kanamycin (30µg), neomycin (30µg), ofloxacin (5µg), penicillin G (10 IU) and tetracycline (30µg) by disc diffusion technique. Results indicated that camel isolates of *S. aureus* were found 100% sensitive to tetracycline. While, ofloxacin and amikacin were found 100% effective against sheep and dog isolates respectively. Whereas, chloramphenicol showed 100% sensitivity against isolates of dog and horse. Ampicillin (0% sensitive to buffalo, cattle, goat, dog and horse isolates) and sulfanilamide (0% sensitive to buffalo, cattle, camel, goat and sheep isolates) showed least sensitivity as compared to other antimicrobial agents. However, poultry isolates showed least, while camel and horse isolates showed most response for the antimicrobial agents. These results indicates the host-microbe interaction and also probable role of host in progression of resistance in *S. aureus* against antimicrobial agents.

Keywords | *Staphylococcus aureus*, Antimicrobial susceptibility, Animals, Pakistan

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is known as anaerobic, Gram negative cocci that commonly causes infections in humans and food animals. It is found as a normal bacterial flora of skin and mucosal surfaces of the respiratory, upper alimentary and urogenital tract. It is known as one of the most prevalent and clinically significant pathogens worldwide. It causes a variety of illnesses ranging from superficial skin eruptions to life threatening infections with bacteremia, endocarditis, pneumonia and toxic shock syndrome (Rubin et al., 2011). *S. aureus* is a major cause of morbidity and causes significant economic losses in farm animals. In poultry, it is a main cause of septic arthritis, septicemia and limb infections (Nazia et al., 2015); in dairy animals it is responsible to cause clinical and sub-clinical mastitis (Tyagi et al., 2013); in horses it is reported as caus-

ative agent of dermatitis and septic arthritis (Weese et al., 2006); while in dogs it causes otitis externa and urinary tract infections (Silva et al., 2001). It has also been reported in inflammatory conditions of bones and joints, cutaneous lesions, septicemia, pneumonia, toxic shock syndrome, meningitis and renal impairments, besides food poisoning (Pereira et al., 2011). Since, methicillin-resistant *Staphylococcus aureus* (MRSA) was first identified in 1961, it has become the most common cause of nosocomial and community infections worldwide (Deresinski, 2005).

A wide array of antibacterial agents are being used to treat bacterial infections including those caused by *S. aureus*. The indiscriminate use of antibiotics particularly in livestock industry as prophylactic and growth promoting agents results the emergence of antibiotic resistant bacteria (Ansari et al., 2014). Resistance to commonly used antimicrobials

is frequently encountered with *S. aureus*. A shift in resistant or sensitivity pattern from time to time in a particular area is well established. Moreover, resistance level is largely variable from area to area (Rubin et al., 2011). Therefore for rationale use of antibiotics there is a great need to explore the antibiotic sensitivity profile of important pathogens on periodical basis. The objective of this study was to determine *in vitro* antimicrobial sensitivity profile of an archived collection of *S. aureus* isolates recovered from different animal species in Hyderabad, Pakistan. This study, additionally will illuminate the concepts of host-microbe interaction for development of antimicrobial resistance.

MATERIALS AND METHODS

BACTERIAL ISOLATES

Staphylococcus aureus isolates (n=80) were recovered from adult animal species including sheep, goat, buffalo, camel, horses, cattle, dog and human. These were isolated from various types of samples such as blood, pus, milk, injuries, and surgical and non-surgical wounds. The samples were collected from Hyderabad, a second largest city of Sindh province of Pakistan. All Samples were collected in sterilized bijoux bottles under strict aseptic conditions and transported to the laboratory in a refrigerated container. These were streaked onto nutrient agar, MacConkey's agar and blood agar with sterilized platinum loop and incubated at 37°C for overnight. The detailed identification of the *S. aureus* isolates were done as described by Malik (1986). Streak plate method was adopted to obtain distinct colonies. *S. aureus* were identified on the basis of cultural characteristics, colony morphology, Gram's staining behavior, and biochemical tests including fermentation of sugars and production of coagulase. After identification, isolates were snap frozen in skim milk at -80°C until analyzed for *in vitro* antimicrobial susceptibility.

IN VITRO ANTIMICROBIAL SUSCEPTIBILITY TESTING

In vitro antimicrobial susceptibility testing for eleven different antibiotics was performed using the disk diffusion method as described by NCCLS (1993). For this purpose, Muller Hinton agar (Difco laboratories, USA) was prepared and dried by incubating at 37°C for 30 minutes. Commercially prepared discs (Becton, Dickinson, Cockeysville, MD, USA) for the following antimicrobials were used: ampicillin (10µg), amikacin (30µg), chloramphenicol (30µg), sulfanilamide (30µg), erythromycin (15µg), gentamicin (10µg), kanamycin (30µg), neomycin (30µg), ofloxacin (5µg), penicillin G (10 IU) and tetracycline (30µg). Drug dosages were chosen as described by Abo-State et al. (2012). Discs were placed over the surface of agar plate with the help of disc dispenser and slightly pressed with sterile forceps to make it adhere to the surface of the medium. The plates were closed, wrapped in aluminum foil, inverted (medium up and disc downward) and incubated

for 24h at 37°C. The zones of inhibition for antimicrobial discs were observed and recorded with annotations.

RESULTS

SUSCEPTIBILITY PROFILE OF *S. aureus* ISOLATED FROM BUFFALO

During present study, buffalo isolates of *S. aureus* were found highly sensitive to ofloxacin, erythromycin and chloramphenicol and their efficacy was recorded as 86.67, 86.67 and 80.00% respectively (Table 1). Whereas, gentamicin and amikacin were found less effective against *S. aureus* as 60.00 and 53.33 % respectively. However, ampicillin and sulfanilamide showed complete resistance (0% sensitive) against the isolates.

SUSCEPTIBILITY PROFILE OF *S. aureus* ISOLATED FROM CATTLE

As shown in Table 1, the erythromycin and chloramphenicol were found as most effective drugs against the *S. aureus* isolates of cattle, and their efficacy was recorded as 86.67 and 80.00% respectively. While quite effective antibiotics against *S. aureus* recorded were gentamicin, ofloxacin and amikacin and their levels of efficacy against the organism recorded were 60.00, 60.00 and 53.33% respectively. Ampicillin and sulfanilamide were found as a least effective drug (0% sensitive) against the isolates of cattle.

SUSCEPTIBILITY PROFILE OF *S. aureus* ISOLATED FROM CAMEL

S. aureus isolated from the samples of camels was tested for susceptibility to various antibiotics and found highly sensitive (100.00%) to tetracycline (Table 1). Whereas, the second most highly effective drugs against the organism noted were ofloxacin, neomycin, kanamycin and amikacin, and their efficacy was recorded as 85.70%. Overall, camel isolates showed highest sensitivity for all the antimicrobial agents as compared to isolates of other animal species.

SUSCEPTIBILITY PROFILE OF *S. aureus* ISOLATED FROM GOAT

The susceptibility to various antibiotics against *S. aureus* isolated from goats was analyzed and results were summarized in Table 1. It was found that highly effective drugs against the isolate were: erythromycin (75.00%), gentamicin (75.00%), neomycin (70.00%) and penicillin G (60.00%). However, quite effective antibiotics against *S. aureus* noted were kanamycin (50.00%) and chloramphenicol (45.00%).

SUSCEPTIBILITY PROFILE OF *S. aureus* ISOLATED FROM SHEEP

As shown in 1 Table, sheep isolates of *S. aureus* were highly sensitive to ofloxacin, tetracycline, gentamicin, ampicillin,

Table 1: The *in vitro* antimicrobial sensitivity (%) of *Staphylococcus aureus* isolated from different animal species

Animal species	Antimicrobial agents*											Overall
	Amp	Ami	Chl	Sul	Ery	Gen	Kan	Neo	Pen G	Tet	Ofi	
Buffalo	0.00	53.33	80.00	0.00	86.67	60.00	33.33	46.67	33.33	46.67	86.67	526.63
Cattle	0.00	53.33	80.00	0.00	86.67	60.00	33.33	46.67	46.67	46.67	60.00	513.30
Camel	71.40	85.70	57.00	0.00	42.80	78.50	85.70	85.70	0.00	100.00	85.70	692.50
Goat	0.00	0.00	45.00	0.00	75.00	75.00	50.00	70.00	60.00	33.33	0.00	408.33
Sheep	61.50	46.10	3.84	0.00	50.00	96.10	23.00	3.84	57.00	73.00	100.00	514.38
Dog	0.00	100.00	100.00	66.67	75.00	0.00	43.75	0.00	33.33	0.00	86.67	505.40
Horse	0.00	66.67	100.00	66.67	73.33	57.00	86.67	86.67	93.33	46.67	0.00	676.96
Poultry	18.75	43.75	50.00	18.75	56.25	33.33	62.50	43.75	33.33	33.33	0.00	393.74
Human	68.70	56.20	0.00	43.75	62.50	81.25	75.00	0.00	33.33	75.00	81.25	576.98
Overall	220.35	505.07	515.84	195.82	608.20	541.18	493.27	383.27	390.31	454.64	500.27	

Amp: Ampicillin; **Ami:** Amikacin; **Chl:** Chloramphenicol; **Sul:** Sulfanilamide; **Ery:** Erythromycin; **Gen:** Gentamicin; **Kan:** Kanamycin; **Neo:** Neomycin; **Pen G:** Penicillin G; **Tet:** Tetracycline; **Ofi:** Ofloxacin

penicillin G and erythromycin, and their susceptibility levels were recorded as 100.00, 73.00, 96.10, 61.50, 57.00 and 50.00% respectively. However, quite effective antibiotic against *S. aureus* noted was amikacin (46.10%), whereas, chloramphenicol and neomycin were found as weakly effective (3.84% sensitivity) against the sheep isolates (Table 1).

SUSCEPTIBILITY PROFILE OF *S. aureus* ISOLATED FROM DOG

The antibiotics, chloramphenicol and amikacin were noted as the highly effective drugs against the isolates of dog and their sensitivity level was recorded as 100.00% (Table 1). Similarly, the efficacy of ofloxacin, erythromycin, sulfanilamide, kanamycin and Penicillin G were recorded as 86.67, 75.00, 66.67, 43.75 and 33.33%, respectively. However, dog isolates of *S. aureus* was found 100.00% resistant to ampicillin, gentamicin, neomycin and tetracycline.

SUSCEPTIBILITY PROFILE OF *S. aureus* ISOLATED FROM HORSES

The bacterial organisms, *S. aureus* isolated from horses were tested for its susceptibility to various antibiotics and results were presented in Table 1. Chloramphenicol and penicillin G were the most effective drugs i.e., highly susceptible and their susceptibility levels were recorded as 100.00 and 93.33% respectively. While, quite effective antibiotics against *S. aureus* isolates of horses noted were kanamycin (86.67%), neomycin (86.67%), erythromycin (73.33%), sulfanilamide (66.67%), amikacin (66.67%), gentamicin (57.00%) and tetracycline (46.67%). Overall, after camel, the horse isolates were found most sensitive for all the antimicrobials tested as compared to isolates of other origins.

SUSCEPTIBILITY PROFILE OF *S. aureus* ISOLATED FROM POULTRY BIRDS

Overall, *S. aureus* isolates of poultry were found least sensitive for the antimicrobial agents (Table 1). Kanamycin,

erythromycin and chloramphenicol were only the antimicrobial agents that showed $\geq 50\%$ sensitivity for the *S. aureus* isolates. However, ofloxacin showed no response (0% sensitivity) for the bacterial organisms.

SUSCEPTIBILITY PROFILE OF *S. aureus* ISOLATED FROM HUMAN BEING

Table 1 indicates that, the *S. aureus* isolates of human being was highly susceptible to ofloxacin (81.25%), gentamicin (81.25%), tetracycline (75.00%), and kanamycin (75.00%). While, ampicillin (68.70%), erythromycin (62.50%), amikacin (56.20%) and sulfanilamide (43.75%) were noted as moderately effective antibiotics against bacterial isolates.

DISCUSSION

A number of pharmacokinetic studies have been performed for *S. aureus* isolated from different animal species, however, to the best of our knowledge this is a first report from Pakistan about an archived collection of *S. aureus* isolated from almost all farm animal species. During present study isolates of *S. aureus* were recovered from different animal species including sheep, goat, buffalo, camel, horses, cattle, dog and human. These were isolated from various types of samples such as blood, pus, milk, injuries, surgical and non-surgical wounds. Eleven different antimicrobial agents i.e., ampicillin (10 μ g), amikacin (30 μ g), chloramphenicol (30 μ g), sulfanilamide (30 μ g), erythromycin (15 μ g), gentamicin (10 μ g), kanamycin (30 μ g), neomycin (30 μ g), ofloxacin (5 μ g), penicillin G (10 IU) and tetracycline (30 μ g) were used to analyze the sensitivity pattern of various *S. aureus* isolates. Results indicated that sulfanilamide and ampicillin showed least sensitivity as compared to other antimicrobials. Similar findings were reported by Matanović et al. (2012) and Alian et al. (2012). Ampicillin is a beta-lactam antibiotic and evidences have suggested that resistance to beta-lactamase sensitive penicillin is ex-

tensive among *S. aureus* regardless of animal origin (Kumar et al., 2013). A recent study have also indicated the high (90.90%) resistance profile of *S. aureus* strains isolated from cattle, goat and sheep for penicillin (Mai-siyama et al., 2014). Sulfonamides are most common antimicrobial agents used in veterinary practices, particularly in developing countries where non judicial uses of antibiotics are very common. It results the development of resistance for sulfonamides and trimethoprim for most of pathogenic bacteria of livestock animals including *S. aureus*. Plasmid pWA2 has been known to cause sulfonamide resistance in *S. aureus* (Then, 1989). Moreover, when little doses of antibiotics were used against microbes, they prevent the growth of susceptible bacteria and leave a small number of resistance bacteria, which propagate efficiently (Farzana et al., 2004).

Adamu et al. (2010) isolated *Staphylococcus aureus* from apparently healthy humans, sheep, goats and cattle. All isolates were tested against 13 antimicrobial agents. Of these, gentamycin, norfloxacin, tetracycline, streptomycin and erythromycin showed the highest activity against *S. aureus* while cefotaxime, ceftazimide and ampiclox showed least activity. The authors concluded that gentamycin, norfloxacin, tetracycline, streptomycin, and erythromycin are the drugs of choice against the infections caused by *S. aureus*. Likewise, Parmar et al. (2014) carried out *in vitro* antimicrobial sensitivity assay of the 35 isolates of *S. aureus* from man, animal and environment origin, and showed the resistance pattern ranging from 14.29 to 60.00%. Tetracycline was found to be the most effective drug (85.72 %) followed by enrofloxacin (71.43 %), ampicillin (71.43 %), gentamicin (71.43 %), ciprofloxacin (48.57 %), co-trimoxazole (48.43 %) and furazolidone (40.00 %). The considerable difference in the susceptibility of the bacterial species to various antibiotics between the present study and that of other work might be due to host species because the host species could play role to increase or decrease the susceptibility of the microorganism to different drugs (Cameron, et al., 2011).

It has been hypothesized that host-pathogen interactions also play a major role in progression of antimicrobial resistance among infectious agents. In order to illuminate these connections research has focused on toxins production, release of extracellular virulence products, adherence to biotic and abiotic surfaces, and phagocytosis. Moreover, functional genomics research have recognized a number of cascade pathways responsible for host-microbe interaction. Some of those have been linked with development of resistance in microbial community for antimicrobial agents (Cameron, et al., 2011; Watkins et al., 2012). Our present results also reflected a probable role of host species in susceptibility/resistance pattern of *S. aureus*. As, poultry isolates showed least, while camel and horse isolates

showed most response for the antimicrobial agents in present study. In agreement to our results, a recent study in The Netherlands have reported that equine isolates were predominantly susceptible to most antimicrobial agents tested, however the human isolates showed least sensitivity. They concluded that no methicillin-resistant *S. aureus* was present in healthy horses whereas methicillin-resistant coagulase negative *Staphylococci* were commonly present (Busscher et al., 2006).

CONCLUSIONS

The results were found highly varied for sensitivity pattern of *S. aureus* isolates of different origins. Ampicillin and sulfanilamide showed least sensitivity as compared to other antimicrobials. However, poultry isolates showed least, while camel and horse isolates showed most response for the antimicrobial agents. These results indicates the probable host-microbe interaction (as also been suggested by previous workers) and also potential role of host in progression of resistance in bacteria against antimicrobial agents. Further studies are warranted to elucidate the exact phenomenon behind the variations in antimicrobial susceptibility of *S. aureus* isolates of different animal origin.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

This work was a part of M. Phil project of first author Fazia Habib. While, Asghar Ali Kamboh and Rahmatullah Rind were the mentors of her project. While, Rehana Burriro and Kanwar Kumar Malhi equally helped in writing and revision of this manuscript.

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