

Investigation of antibiotic resistance among *Staphylococcus aureus* strains of human and bovine origin

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Summary: The objective was to investigate antibiotic resistance patterns of *Staphylococcus aureus* strains isolated from human and bovine by using Kirby-Bauer antibiotic disk test as well as methicillin resistance by using polymerase chain reaction (PCR). Among 114 *S. aureus* strains samples collected from patients hospitalized in various clinics of Harran University Medical School the number and percent of antimicrobial resistant strains were as follows: 114 (100%) ampicillin, 108 (94.7%) penicillin G, 76 (66.6%) rifampin, 75 (65.7%) cefoxitin, 71 (62.2%) cefuroxime, 74 (64.9%) oxacillin, 73 (64%) ciprofloxacin, 74 (64.9%) norfloxacin, 70 (61.4%) gentamycin, 66 (57.8%) imipenem, 64 (56.1%) amoxicillin-clavulanic acid, 61 (53.5%) tetracycline, 37 (32.4%) erythromycin, 38 (33.3%) clindamycin, 11 (9.6%) sulphamethaxazole-trimethoprim and 8 (7%) vancomycin. None of vancomycin resistant *S. aureus* was found by E-test. Among 64 *S. aureus* strains isolated from subclinical bovine mastitis, all were resistant to penicillin and ampicillin while all were found to be highly sensitive to oxacillin, cefoxitin, imipenem, cefuroxime, vancomycin, ciprofloxacin, norfloxacin, rifampin and sulphamethaxazole-trimethoprim. The number and percent of antimicrobial resistance to other antibiotics were as follows: 13 (20%) gentamicine, 6 (9.3%) erythromycin, 5 (7.8%) clindamycin, 4 (6.2%) tetracycline, 1 (1.5%) amoxicillin-clavulanic acid. All strains were resistant to penicillin and ampicillin. PCR analysis showed that 76 (66.6%) of total 114 methicillin resistant *S. aureus* (MRSA) strains of human origin had *mecA* gene. This gene was not detected in bovine strains. In conclusion, the results of the present study indicated that the incidence of antibiotic resistance of *S. aureus* strains isolated from humans was higher than that from cattle, penicillin and ampicillin resistance of *S. aureus* strains of human and cattle origin were highly widespread as well as the methicillin resistance was highly prevalent among *S. aureus* strains of human origin while it was absent or low among those of cattle origin.

Key words: Antibiotic resistance, Cattle, Human, PCR, *Staphylococcus aureus*.

İnsan ve sığır orjinli *Staphylococcus aureus* suşlarının antibiyotik dirençlerinin araştırılması

Özet: Bu çalışmada, insan ve sığırlardan izole edilen *Staphylococcus aureus* suşlarında antibiyotiklere direnç oranının Kirby-Bauer disk difüzyon yöntemiyle araştırılması ve metisilin direncinin PCR yöntemiyle saptanması amaçlandı. Harran Üniversitesi Tıp Fakültesi Hastanesi'nin çeşitli servislerinde yatan hastalardan izole edilen 114 adet *S. aureus* suşunun, 114 (%100)'ü ampisilin, 108 (%94.7)'i penisilin G, 76 (%66.6)'sı rifampin, 75 (%65.7)'i sefoksitin, 74 (%64.9)'ü oksasilin ve norfloksasin, 73 (%64)'ü siprofloksasin, 71 (%62.2)'i sefuroksim, 70 (%61.4)'i gentamisin, 66 (%57.8)'sı imipenem, 64 (%56.1)'ü amoksisillin-klavulanik asid, 61 (%53.5)'i tetrasiklin, 37 (%32.4)'si eritromisin, 38 (%33.3)'i klindamisin, 11 (%9.6)'i sulfametaksazol+trimetoprim ve 8 (%7)'i vankomisine dirençli olarak saptandı. Ancak, vankomisin dirençli *S. aureus* suşlarının hiçbiri E testi ile dirençli bulunmadı. Subklinik inek mastitislerinden izole edilen 64 adet *S. aureus* suşunun 13 (%20)'ü gentamisin, 6 (%9.3)'sı eritromisin, 5 (%7.8)'i klindamisin, 4 (%6.2)'ü tetrasiklin, 1 (%1.5)'i amoksisillin-klavulanik aside ve tamamı penisillin ve ampisilline dirençli bulunurken, oksasillin, sefoksitin, imipenem, sefuroksim, vankomisin, siprofloksasin, norfloksasin, rifampin, sulfametaksazol+trimetoprim tamamen duyarlı olduğu belirlendi. PCR ile insan kaynaklı toplam, 114 metisilin dirençli *S. aureus* (MRSA) suşunun 76 (%66.6)'sında *mecA* geni saptanırken, sığır orjinli suşlarda *mecA* geni saptanamadı. Sonuç olarak, insanlardan izole edilen *S. aureus* suşlarında, antibiyotik direncinin sığırlara göre daha yaygın olduğu, insan ve sığır kaynaklı *S. aureus* izolatlarında penisilin ve ampisilin direncinin yüksek olduğu, insanlarda metisilin direncinin yaygın olduğu ancak sığırlarda metisilin direncinin insanlardaki kadar yaygın olmadığı kanısına varılmıştır.

Anahtar kelimeler: Antibiyotik direnci, İnsan, PCR, Sığır, *Staphylococcus aureus*.

Introduction

Staphylococci are Gram positive bacteria widely spread in nature. These agents possess an opportunistic pathogen character and cause various diseases with different clinical presentations in humans and animals.

They cause various infections such as mastitis, tick fever, periorbital eczema, osteomyelitis, arthritis in domestic animals (1, 23, 24). In humans, staphylococci are frequently isolated from bacterial infections in various regions of the body, particularly in serious and life

threatening infections such as toxic shock syndrome, respiratory system infections, endocarditis, thrombophlebitis, food poisoning, septic arthritis, osteomyelitis, meningitis, sepsis and bacteremia (14, 29).

S. aureus, MRSA isolates in particular, are critical pathogens for both human and animal health. MRSA strains are reported to be contagious between humans and animals (20). In recent years, increase in rate of MRSA infections and resistance against a larger group of antibiotics is striking. Starting from 1970s, MRSA gradually exhibited more resistance to a number of antibiotics which are used widespread in medical practice (30). Due to selection and use of inappropriate antibiotics, high level and wide spectrum resistance develops in these microorganisms and hence, MRSA infections are found to cause significant issues among animals and humans (19, 20).

The purpose of this study, were to investigate methicillin resistance among *S. aureus* strains isolated from human and bovine samples and to determine the level of resistance in isolated strains against different antibiotics.

Materials and Methods

Bacterial Strains: A total of, 178 *S. aureus* strains isolated from milk of cows with subclinical mastitis and from patients hospitalized in various clinics of Harran University Medical School were used.

Antibiotic susceptibility test: *S. aureus* isolates were evaluated by Kirby Bauer disc diffusion method (4) in compliance with standards of Clinical and Laboratory Standards Institute (CLSI) (7). In antibiotic susceptibility test, a total of 16 different antibiotic discs soaked with oxacillin (1 µg), cefoxitin (30 µg), gentamycin (10 µg), imipenem (10 µg), cefuroxime (30 µg), vancomycin (30 µg), erythromycin (15 µg), clindamycin (2 µg), amoxicillin-clavulanic acid (30 µg), ampicillin (10 µg), penicillin G (10 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), rifampin (5 µg), tetracycline (30 µg) and sulphamethoxazole+trimethoprim (25 µg) (Oxoid) were used.

Those isolates which were erythromycin resistant were further subjected to D-test according to CLSI guidelines (7). Briefly, erythromycin (15 µg) disc was placed at a distance of 15 mm (edge to edge) from clindamycin (2 µg) disc on a Mueller Hinton agar plate previously inoculated with 0.5 McFarland bacterial suspension. Following an overnight incubation at 37 °C, flattening of zone (D shaped) around clindamycin in the area between the two discs, indicated inducible clindamycin resistance. Resistance to vancomycin was additionally checked by E-test (AB biodisk, Sweden) according to the manufacturer's instructions.

Methicillin Resistance: Methicillin resistance among isolated agents was determined both phenotypically and genotypically.

Phenotypic Method: Methicillin resistance was phenotypically determined by disc diffusion method through utilization of cefoxitin (30 µg) and oxacillin (1 µg) discs in compliance with protocols of CLSI (7).

Genotypic method: DNA isolation from isolated *S. aureus* isolates was performed by using phenol-chloroform method according to Sambrook et al. (25). Isolated DNAs were kept at -20°C until time of assay. For the purpose of amplification, PCR reaction mixture was prepared by modifying the method reported by Choi et al. (6). Reaction mixture composed of 4 µl MgCl₂, 0.5 µl dNTP mixture, 1 µl Met1, (CCT AGT AAA GCT CCG GAA), 1 µl Met2 (CTA GTC CAT TCG GTC CA), 2.5 µl PCR buffer, 0.2 µl Taq DNA polymerase, and sterile distilled water to yield a final concentration of 25 µl. 2 µl template DNA was added to mixture. As positive control a methicillin-resistant *S. aureus* strain (ATCC 95047) was used. As negative control a *mecA*-negative strain (ATCC 29213) was used. PCR conditions, consisted of an initial denaturation of 5 minutes at 95 °C, followed by 30 cycles of a denaturation step for 2 minutes at 95 °C, annealing for 30 seconds at 58 °C and extension for 30 seconds at 72 °C. Amplified samples obtained at PCR were subjected to 2% agarose gel electrophoresis stained by ethidium bromide and visualized by using a UV Transilluminator (6).

Results

Among 114 *S. aureus* isolates isolated from humans, resistance was detected in 114 (100%) samples to ampicillin, in 108 (94.7%) to penicillin G, in 76 (66.6%) to rifampin, in 75 (65.7%) to cefoxitin, in 74 (64.9%) to oxacillin, in 73 (64%) to ciprofloxacin, in 74 (65%) to norfloxacin, in 71 (62.2%) to cefuroxime, in 70 (61.4%) to gentamycin, in 66 (57.8%) to imipenem, in 64 (56.1%) to amoxicillin-clavulanic acid, in 61 (53.5%) to tetracycline, in 37 (32.4%) to erythromycin, in 38 (33.3%) to clindamycin, in 11 (9.6%) to sulphamethoxazole+trimethoprim and in 8 samples (7%) to vancomycin. By using D-test 11 strains detected to be resistant to clindamycin. Vancomycin resistant strains gave negative results in E-test. Among resistant samples, resistance was detected against one antibiotic in 5 strains (4.3%) and multiple antibiotic resistance was observed in 109 samples (95.6%) (two antibiotics in 22 samples, three in 8, four in 2 and five or more antibiotics in 82 samples) (Table 1).

Among 64 *S. aureus* isolates obtained from subclinic bovine mastitis, resistance was determined in 13 (20%) samples against gentamycin, in 6 (9.3%) against erythromycin, in 5 (7.8%) against clindamycin, in 4 (6.2%) against tetracycline, in 1 (1.5%) sample against amoxicillin-clavulanic acid and all samples were resistant against penicillin and ampicillin, whereas all strains were susceptible to oxacillin, cefoxitin, imipenem, cefuroxime,

Tablo 1. *S. aureus* suşlarının antibiyotik duyarlılık ve dirençliliklerinin dağılımı.
Table 1. Distribution of antibiotic susceptibility and resistance among *S. aureus* strains.

Antibiotics	Human strains n:114			Bovine strains n:64		
	Resistant (R) N (%)	Intermediate (I) N (%)	Susceptible (S) N (%)	Resistant (R) No (%)	Intermediate (I) N (%)	Susceptible (S) N (%)
Oxacillin (1 µg)	74 (64.9%)	0 (0%)	40 (35%)	0 (0%)	0 (0%)	64 (100%)
Cefoxitin (30 µg)	75 (65.7%)	1 (0.8%)	38 (33.3%)	0 (0%)	0 (0%)	64 (100%)
Gentamycine (10 µg)	70 (61.4%)	1 (0.8%)	43 (37.7%)	13 (20%)	0 (0%)	51 (79.6%)
Imipenem (10 µg)	66 (57.8%)	0 (0%)	48 (42.1%)	0 (0%)	0 (0%)	64 (100%)
Cefuroxime (30 µg)	71 (62.2%)	0 (0%)	43 (37.7%)	0 (0%)	0 (0%)	64 (100%)
Vancomycin (30 µg)	8 (7%)	0 (0%)	106 (93%)	0 (0%)	0 (0%)	64 (100%)
Erythromycin (15 µg)	37 (32.4%)	7 (6.1%)	70 (61.4%)	6 (9.3%)	0 (0%)	58 (90%)
Clindamycin (2 µg)	38 (33.3%)	3 (2.6%)	73 (64%)	5 (7.8%)	0 (0%)	59 (92.1%)
Amoxicillin-clavulanic Acid (30 µg)	64 (56.1%)	0 (0%)	50 (43.8%)	1 (1.5%)	0 (0%)	63 (98.5%)
Ampicillin (10 µg)	114 (100%)	0 (0%)	0 (0%)	64 (100%)	0 (0%)	0 (0%)
Penicillin G (10 U)	108 (94.7%)	0 (0%)	6 (5.2%)	64 (100%)	0 (0%)	0 (0%)
Ciprofloxacin (5 µg)	73 (64%)	1 (0.8%)	40 (35%)	0 (0%)	0 (0%)	64 (100%)
Norfloxacin (10 µg)	74 (64.9%)	3 (2.6%)	37 (32.4%)	0 (0%)	0 (0%)	64 (100%)
Rifampin (5 µg)	76 (66.6%)	0 (0%)	38 (33.3%)	0 (0%)	0 (0%)	64 (100%)
Tetracycline (30 µg)	61 (53.5%)	16 (14%)	37 (32.4%)	4 (6.2%)	0 (0%)	60 (93.7%)
Sulphamethoxazole-trimethoprim (25 µg)	11 (9.6%)	1 (0.8%)	102 (89.4%)	0 (0%)	0 (0%)	64 (100%)

Tablo 2. MRSA suşlarının antibiyotik duyarlılık ve dirençliliklerinin dağılımı.
Table 2. Distribution of antibiotic susceptibility and resistance among MRSA.

Antibiotics	Human strains n:76		
	Resistant (R) N (%)	Intermediate (I) N (%)	Susceptible (S) N (%)
Oxacillin (1 µg)	74 (97.3%)	0(0%)	2 (2.6%)
Cefoxitin (30 µg)	75 (98.6%)	0 (0%)	1 (1.3%)
Gentamycine (10 µg)	69 (90.7%)	1 (1.3%)	6 (7.8%)
Imipenem (10 µg)	65 (85.5%)	0 (0%)	11 (14.4%)
Cefuroxime (30 µg)	70 (92.1%)	0 (0%)	6 (7.8%)
Vancomycin (30 µg)	7 (9.2%)	0 (0%)	69 (90.7%)
Erythromycin (15 µg)	33 (43.4%)	4 (5.2%)	39 (51.3%)
Clindamycin (2 µg)	33 (43.4%)	1 (1.3%)	42 (55.2%)
Amoxicillin-Clavulanic Acid (30 µg)	63 (82.8%)	0 (0%)	13 (17.1%)
Ampicillin (10 µg)	76 (100%)	0 (0%)	0 (0%)
Penicillin G (10 U)	76 (100%)	0 (0%)	0 (0%)
Ciprofloxacin (5 µg)	70 (92.1%)	0 (0%)	6 (7.8%)
Norfloxacin (10 µg)	66 (86.8%)	3 (3.9%)	7 (9.2%)
Rifampin (5 µg)	73 (96%)	0 (0%)	3 (3.9%)
Tetracycline (30 µg)	54 (71%)	11 (14.4%)	11 (14.4%)
Sulphamethoxazole+Trimethoprim (25 µg)	9 (11.8%)	1 (1.3%)	66 (86.8%)

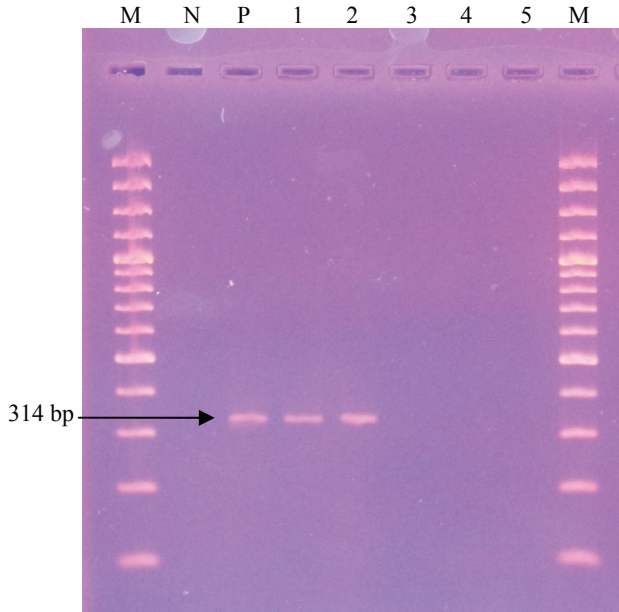
vancomycin, ciprofloxacin, norfloxacin, rifampin, sulphamethoxazole+trimethoprim (Table 1).

Among 114 *S. aureus* strains of human origin, methicillin resistance was determined by cefoxitin and oxacillin disc diffusion methods in 75 (65.7%) and in 74 (64.9%) samples, respectively. Among 114 *S. aureus* strains of human origin evaluated by using PCR, presence of *mecA* gene was determined in 76 samples

(66.6%) thereby were considered MRSA (Figure 1). No *mecA* gene was detected in bovine strains.

Distribution of methicillin resistant *S. aureus* strains evaluated according to presence of *mecA* gene, is shown in Table 2. Among 76 (66.6%) MRSA strains resistance was detected in 75 (98.6%) samples against cefoxitin, in 74 (97.3%) against oxacillin, in 69 (90.7%) against gentamycine, in 65 (85.5%) against imipenem, in 70

(92.1%) against cefuroxime, in 7 (9.2%) against vancomycin, in 33 (43.4%) against erythromycin, in 33 (43.4%) against clindamycin, in 63 (82.8%) against amoxicillin-clavulanic acid, in 76 (100%) against ampicillin and penicillin G, in 70 (92.1%) against ciprofloxacin, in 66 (86.8%) against norfloxacin, in 73 (96%) against rifampin, in 54 (71%) against tetracycline and in 9 samples (11.8%) against sulphamethoxazole+trimethoprim. All *S. aureus* strains resistant to methicillin were determined to exhibit resistance against multiple antibiotics.



Şekil 1- PZR Ürünlerinin Agaroz Jeldeki Görüntüsü. M- 100 bp marker. N, *mecA*-negatif, *S. aureus* (ATCC 29213) P- *mecA*-pozitif, *S. aureus* ATCC (95047) suşu 1-2 *mecA*(+) suslar 3-4-5- *mecA*(-) suslar.

Figure 1- Appearance of PCR products in agarose gel. M, 100 bp marker. N, *mecA*-negative *S. aureus* (ATCC 29213) P- *mecA*-positive, *S. aureus* ATCC (95047) strain 1-2 *mecA*(+) strains 3-4-5- *mecA*(-) strains.

Discussion and Conclusion

Staphylococci are opportunistic pathogens which cause serious infections in animals and humans. They are leading causes of mastitis in dairy cows worldwide. They cause nosocomial and community acquired infections in humans (14). In recent years, a striking increase in the rate of *MRSA* infections has been observed (30).

In different studies the prevalence of *MRSA* strains has been reported as 66.7% in Konya (27), as 82.0 - 88.0% in Gaziantep, (21, 32) and 89.3% in Ankara (22). The prevalence of *MRSA* strains in the present study was similar to that reported by other research groups (21, 22, 27, 32). High prevalences of penicillin (94.7%) resistant strains found in the present study were similar to that (96.5%) reported by Hasbek et al. (13). Resistance rates against TMP-SMX were in accordance with the findings (4.4%) of Arıkan et al. (2) and İnan et al. (16.3%) (15).

In the present study, resistance rates against other antibiotics among methicillin susceptible strains were generally observed to be low whereas resistance rates in methicillin resistant strains were determined to be higher. Sultan et al. (26) and Birengel et al. (5) have also reported similar results. High resistance rates observed in these study, presenting as multiple antibiotic resistance are in accordance with our study.

In animals, the first *MRSA* strain was isolated from bovine with mastitis in 1972 (8). Currently, *MRSA* isolation in animals is reported to be gradually increased (19). In Turkey Güler et al. (11) have reported a resistance rate of 63% against penicillin and ampicillin, 27.9% against oxytetracycline and 1.8% against trimethoprim-sulfamethoxazole while no resistance was detected against amoxicillin-clavulanate, oxacillin, enrofloxacin and kanamycine-cephalexin. Prevalence of methicillin resistant strains of bovine origin in Turkey have been reported to be 18.0% by Hadimli et al. (12), 8.7% by Kireççi and Çolak (18) and 10.4% by Kaynarca and Türkyılmaz (16). Yoshida et al. (31) have a reported methicillin resistance rate of 5.0% among staphylococcus strains obtained from mastitis cases in Japan. In the current study, no methicillin resistance was determined among a total of 64 *S. aureus* strains of bovine origin. Results of study indicated no methicillin resistance or low levels of resistance and these findings are in accordance with our results (9, 10, 11).

In a study conducted in Aydın region, west of Turkey, susceptibility for ciprofloxacin (100%), for each of neomycin, bacitracine, tetracycline (100%), kanamycine (85%) and amoxicillin-clavulanic acid (84%) and resistance against penicillin (95%) and oxacilline (60%) were determined among isolated *S. aureus* strains (17). In a study conducted by Aydın et al. (3) in the city of Kars, 82% of penicillin resistance, 67% of tetracycline resistance and 10% of ciprofloxacin resistance was observed in *S. aureus* strains. Hadimli et al. (12) reported a 61.7% penicillin resistance in a study conducted in Konya. Ünal and İstanbulluoğlu (28) have observed resistance rates of 80.4%, 26.1% and 4.3% against penicillin, tetracycline and erithromycine respectively. while all isolates have been reported to be susceptible for gentamycine, enrofloxacin, rifampine, trimethoprim-sulphamehoxazole, oxacilline, vancomycin, cephalotine and linezolid (28). High penicillin resistance rate observed in the present study suggested a frequent use of penicillin preparations resulting in an increase in the prevalence of β -lactamase producing *S. aureus* enzymes.

In conclusion, the results of the present study indicated that the incidence of antibiotic resistance of *S. aureus* strains isolated from humans was higher than that from cattle, penicillin and ampicillin resistance of *S. aureus* strains of human and cattle origin were highly widespread as well as the methicillin resistance was

highly prevalent among *S. aureus* strains of human origin while it was absent or low among those of cattle origin.

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