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Characteristics of *Staphylococcus aureus* Strains Isolated from Human and Animal Sources

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Abstract: A total of 409 samples were investigated bacteriologically to detect the occurrence of staphylococci among the diseased animals and human, the highest isolation rate was observed in human samples (36%) followed by dog (28%), bovine (24.8%), ration (14.7%) and chicken (12%) samples. A total of 78 *S. aureus* isolates secured from different animals and human origins were characterized and identified using the most important conventional biochemical tests as anaerobic glucose fermentation, catalase, coagulase, acetone production, novobiocin sensitivity and mannitol fermentation. SpA was extracted from 17 *S. aureus* isolates (5 bovine, 6 human, 2 dog, 2 concentrated ration and 2 chicken isolates) as well as from Cowan 1 strain to estimate the molecular size of SpA by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. As well as antibiogram patterns of 15 *S. aureus* isolates have been fully studied.

Key words: Staphylococci % *S. aureus* % SpA

INTRODUCTION

Staphylococci often represent part of normal bacterial flora of the skin and mucosal surfaces of the respiratory, upper alimentary and urogenital tract of mammals and birds. Thus staphylococci are easily spread between animals and under certain conditions to humans as well as by skin to skin contact, but also by contact with excretions which contain staphylococci, such as saliva, or aerosols released during sneezing and coughing. Moreover, staphylococci may be spread by animal products, such as non-pasteurized milk [1]. *S. aureus* causes disease problems such as septicemia and skeletal infections in commercial broiler chicken [2]. Although the mechanism of spread of *S. aureus* infection through poultry flocks is not fully understood. Thompson *et al.* [3] suggested that the hatchery played an important role in the spread of infection to rearing farms. Kawasaki disease (KD) is an acute illness of early childhood characterized by fever, indurations and erythema of the hands and feet, inflammation of the mucous membranes, polymorphous skin rash and cervical lymphadenopathy. *S. aureus*, isolated from the rectum or pharynx of patients with KD, secretes toxic shock syndrome toxin 1 (TSST-1). The KD isolates express low levels of other exoproteins compared to isolates from patients with toxic shock syndrome (TSS) [4]. *S. aureus* is a bacterial pathogen in a variety of infectious diseases in both humans and animals. Many extra cellular proteins and toxins are produced which probably contribute to the virulence of this organism, but the exact role of the potential virulence determinants in different types of infections remains unclear [5]. It is a tantalizing possibility that the expression of high levels of extra cellular SpA, secreted locally by *S. aureus* isolates colonizing the gastrointestinal tract of patients, may contribute to the symptoms of KD. It has been recently demonstrated that, in addition to exhibiting immunoregulatory activities, SpA has the ability to bind to von Willebrand factor, a protein that has a central role in hemostasis and thrombogenesis [4] Serious *S. aureus* infections can be caused by strains that are methicillin-resistant (MRSA) or -susceptible and which may or may not express the pathogenic Panton–Valentine leucocidin (PVL) toxin, also, MRSA are cross-resistant to all currently licensed $-lactam antibiotics [6]. A reliable and rapid identification of *S. aureus* colonies from samples is a cornerstone in the control of *S. aureus* infection. Identification of bacterial pathogens still relies mainly on phenotypic criteria.

The present work was planned to identify and characterize *S. aureus* isolates collected from human and animal samples.

MATERIALS AND METHODS

Samples: A total of 409 samples were collected from cattle, dogs, buffaloes, poultry and concentrated ration for isolation of *Staphylococcus* species. As well as

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Table 1: Types and numbers of the samples collected

Source of the isolates	Type of samples	No. of The examined samples
Bovine	- Milk from mastitic cows	150
	- Milk from mastitic Buffaloes	50
	- Swabs from cow septic wounds	50
Chicken	Swabs from musculoskeletal abscesses	50
Concentrated ration	- Local concentrated rations	20
	- Imported concentrated rations	14
Dog	- Internal organs (Liver – spleen – lung)	25
Human	- Urine from infected urinary tract	20
	- Swabs from septic wounds	20
	- Nasal swabs from cases with respiratory symptoms	10
Total		409

50 human samples were collected from urine and septic wounds obtained from clinics as shown in Table 1.

Isolation and Cultivation of Staphylococci: The collected samples were cultured onto nutrient agar (Difco)sheep blood agar and Bacto-Mannitol salt agar. The inoculated plates were incubated for 24-48 hours at 37°C. Suspected isolates were identified primarily as Gram-positive, catalase positive cocci. The suspected colonies were picked up and propagated in nutrient agar slope for further examinations

Identification and Characterization of *S.aureus*: The isolates were identified according to Cruickshank *et al.* [7] and Quinn *et al.* [8] by using*:* catalase test, coagulase test, mannitol fermentation activity, pigment production onto nutrient agar, hemolytic activity on sheep and human blood agar, DNase activity, lysozyme activity, gelatinase activity, growth on Baird-Parker Medium, lipase activity on egg yolk agar medium, protease activity on milk agar medium, fibrinolysin activity on plasma agar medium, Crystal violet agar growth type [9] and detection of SpA by SpA agglutination kits (Wellcome Diagnostics). Cowan I strain of *S. aureus* was used throughout the investigations for the preparation of the typing coagglutinating reagent and as a positive control for production of cell pound SpA. It was obtained in freeze lyophilized dried ampoules from the Namru 3 in Egypt.

Determination of the Profile of SpA Extracted from *S. aureus* Isolates by Sodium Dodecyl Sulfate Poly Acrylamide Gel Electrophoresis (SDS-Page): to obtain the analytical profile of SpA extracted from the isolates according to Laemli [10]. Preparation of protein A from *S. aureus* was carried out according to Kessler [11].

Table 2: Incidence of staphylococci among the examined samples

Source of the isolates	No. of the examined samples	*Staphylococcus* species	
		No.	%
Bovine	250	62	24.8
Chicken	50	6	12.0
Concentrated Ration	34	5	14.7
Dog	25	7	28.0
Human	50	18	36.0
Total	409	98	23.9

No. Number of Positive

% was calculated according to the number of the examined samples of each type

Determination of Susceptibility of *S. aureus* Isolates to 15 antibacterial agents: (amoxycillin, amoxycillin/ clavulanic acid, ampicillin, cefoperazone, cefotaxime, ciprofloxacin, enroflaxacin, gentamicin, methicillin, neomycin, ofloxacin, oxytetracycline, streptomycin, sulfamethoxazole/ trimethoprim and tobramycin disks). The disk diffusion technique was adapted according to Finegold and Martin [12]. Interpretation of the results was adapted according to NCCLS [13].

RESULTS AND DISCUSSION

Infections due to staphylococci are of major importance to veterinary and human medicine. *S. aureus* is one of the most significant pathogens causing intramammary infections in dairy cattle world wide [14]. *S. aureus* is the leading cause of nosocomial infections and is responsible for a wide range of human diseases, including endocarditis, food poisoning, toxic shock syndrome, septicemia, skin infections, soft tissue infections and bone infections, as well as bovine and ovine mastitis [15].

A total of 409 samples were investigated bacteriologically to detect the occurrence of staphylococci among the diseased animals and human. As shown in Table 2, the highest isolation rate was observed in human samples (36%) followed by dog (28%), bovine (24.8%), ration (14.7%) and chicken (12%) samples. In this concern, Joklik *et al*. [16] stated that staphylococci were responsible for over 80 percent of the suppurative diseases encountered in medical practice. They added that they cause most suppurative infections of the skin but may also invade and produce severe infections.

Table 3: Prevalence of *Staphylococcus* species from the collected samples

		Staphylococcus species					
		S. aureus		*S. intermedius*		*S. hyicus*	
Source of the isolates	No. of the examined samples	No.	%	No.	%	No.	%
Bovine	250	53	21.2	6	2.4	3	1.2
Chicken	50	4	8.0	2	4.0	-	-
Concentrated Ration	34	3	8.8	1	2.9	1	2.9
Dog	25	4	16.0	3	12.0	-	-
Human	50	14	28.0	3	6.0	1	2.0
Total	409	78	19.1	15	3.7	5	1.2

Table 4: Prevalence of *S. aureus* from the collected samples

			S. aureus	
Source of the isolates	Type of samples	No. of The examined samples	No.	%
Bovine	- Milk from mastitic cows	150	34	22.7
	- Milk from mastitic Buffaloes	50	8	16.0
	- Swabs from cow septic wounds	50	11	22.0
Chicken	Swabs from musculoskeletal abscesses	50	4	8.0
Concentrated ration	- Local concentrated rations	20	2	10.0
	- Imported concentrated rations	14	1	7.1
Dog	- Internal organs (Liver – spleen – lung)	25	4	16.0
Human	- Urine from infected urinary tract	20	3	15.0
	- Swabs from septic wounds	20	9	45.0
	- Nasal swabs from cases with respiratory symptoms	10	2	20.0
Total		409	78	19.1

No. Positive number

% was calculated according to the number of the examined samples of each type.

The investigators reported that the production of coagulases and thermonucleases are not unique features of *S. aureus* but are shared by *S. intermedius* and *S. hyicus*. As shown in Table 3 *S. aureus*, *S. intermedius* and *S. hyicus* were identified from the examined samples with percentage 19.1, 3.7 and 1.2 respectively. In veterinary medicine, three staphylococcal species are of particular importance as a primary cause of specific diseases: *S. aureus* (mastitis in ruminants, equine botryomycosis), *S. hyicus* (porcine exudative epidermitis) and *S. intermedius* (canine pyoderma), as recorded by Euzeby [17]. *S. intermedius* is considered the primary cause of canine and also feline pyoderma. Chronic and recurrent pyoderma – often seen in dogs – is considered a complex syndrome in which not only the staphylococci, but also cell-mediated hypersensitivity, endocrine disorders and a genetic predisposition may play an important role in the development of the disease. In dogs and cats, *S. intermedius* has also been reported by Werckenthin *et al*. [1] to be involved in other diseases, such as pyometra, otitis externa and purulent infections of the joints, eyelids and conjunctiva.

Data present in Table 4 concluded that *S. aureus* could be identified from human septic wound (45%), nasal swabs from diseased human (20%) and urine collected from diseased human (15%). Humans are known to carry *S. aureus* in their anterior nares, with a mean carriage rate of 37.2% in the general population [18]. Nasal carriage by humans has been shown to be important in the epidemiology of disease associated with *S. aureus* in humans and the nasal cavity may act as a reservoir for the infection of other sites around the body.

S. aureus was isolated from mastitic cow (22.7%) and buffaloes (16%), as well as from cattle septic wounds (22%) as shown in Table 4.

Table 5: Collective table shows the characteristic features of the examined *S. aureus* isolates

Source of the Isolates	No. of examined samples	No. of *S.aureus* isolates	Colony pigment: White No.	%	Creamy No.	%	Golden yellow No.	%	Hemolytic activity: Sheep blood agar No.	%	Human blood agar No.	%	Non hemolytic No.	%	Dnase activity No.	%	Lysozyme activity No.	%	Gelatinase activity No.	%	Lecithinase activity No.	%	Lipase activity No.	%
Bovine	250	53	2	3.8	16	30.2	35	66.0	48	91	11	20.8	5	9	36	67.9	49	92.5	49	92.5	43	81.1	25	47.2
Chicken	50	4	1	25	1	25	2	50	3	75	1	25	1	25	2	50	3	75	3	75	3	75	2	50
Concentrated rations	34	3	-	0	1	33.3	2	66.7	3	100	1	33.3	-	0	2	66.7	3	100	3	100	2	66.7	1	33.3
Dog	25	4	-	0	1	25	3	75	3	75	-	0	1	25	3	75	3	75	4	100	3	75	1	25
Human	50	14	2	14.3	4	28.6	8	57.1	13	92.9	2	14.3	1	7.2	13	92.9	14	100	13	92.9	11	78.6	12	85.7
Total	409	78	5	6.4	23	29.5	50	64.1	70	89.7	15	19.2	8	10.3	56	71.8	72	92.3	72	92.3	62	79.5	41	52.6

Table 5: Continue

Protease activity No.	%	Tellurite reduction No.	%	Fibrinolysin No.	%	SpA by agglutination No.	%	Crystal violet medium: Yellow(A) No.	%	Violet(C) No.	%	White(E) No.	%	Mannitol No.	%	Novobiocin (S) No.	%	Acetone production No.	%	SDS
39	73.6	51	96.2	40	75.5	39	73.6	13	24.5	33	62.3	7	13.2	53	100	5/5	100	53	100	(5*) 44.4-45.9kD
2	50	3	75	1	25	1	25	2	50	2	50	0	0	4	100	2/2	100	4	100	(2*) 46**kDa
2	667	3	100	2	66.7	1	33.1	1	33.3	2	66.7	0	0	3	100	2/2	100	3	100	(2*) 45.7** kDa
2	50	4	100	2	50	3	75	1	25	2	50	1	25	4	100	2/2	100	4	100	(2*) 45.46 kDa
12	85.7	14	100	14	100	12	85.7	9	64.3	4	28.6	1	7.1	14	100	6/6	100	14	100	(6*) 45 kDa
57	73.1	75	96.2	59	75.6	56	71.8	26	33.3	43	55.1	9	11.6	78	100	17/17	100	78	100	(16*) 44.6 kDa

No. Positive number, % was calculated according to the number of *S. aureus*

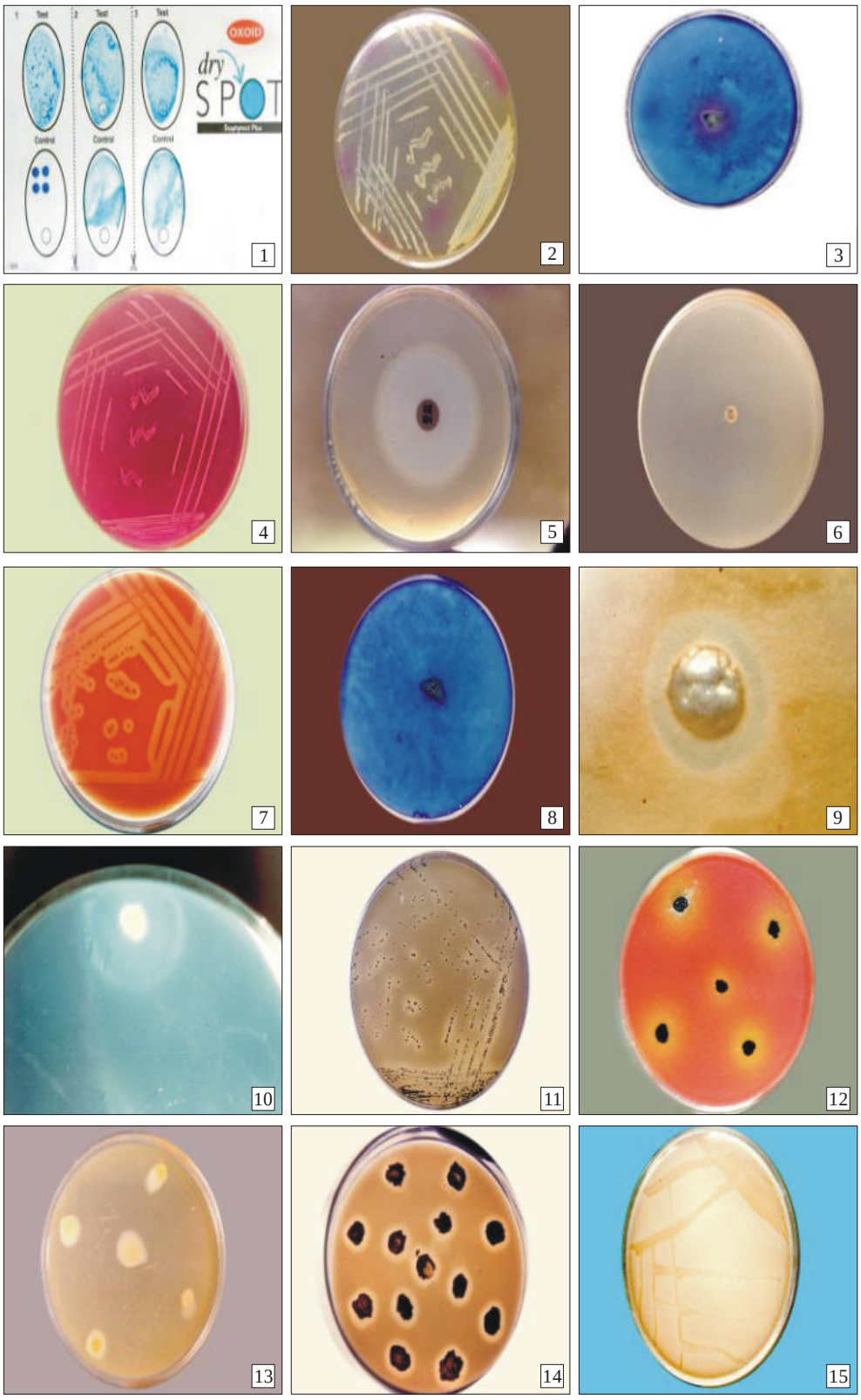
* number of *S. aureus* isolates examined ** one isolate only had SpA

Ekman *et al*. [19] concluded that, different microbes evoke different inflammatory responses due to different virulence factors, Gram positive bacteria, mainly staphylococci and streptococci, cause about 64% of the clinical cases of mastitis and the dominating microbe is *S. aureus*.

A total of 78 *S. aureus* isolates secured from different animals and human origins were characterized and identified using the most important conventional biochemical tests according to the proposed scheme of Quinn *et al*. [8]. The routinely used coauglase, acetoin production from glucose, novobiocin sensitivity and mannitol fermentation were uniformly positive in these isolates as shown in photos(1-5). Overall analysis of data collected in Table 5 and photos (1-18) revealed that, *S. aureus* produced many extracellular products which are considered as possible virulence factors. Among bovine isolates, 96.2% of the isolates reduced tellurite, 92.5% of the isolates produced lysozyme and gelatin (each), 91% had hemolytic activity on sheep RBCs, 81.1% had lethicinace, 75.5% produced fibrinogen, 73.6% produced protease and SpA by agglutination test (each), 67.9% produced DNase, 66.1% had golden yellow endopigment and 62.3% of the isolates had violet growth on crystal violet agar.

The strains which infected the dairy herd studied by Laevens *et al*. [20] had negative clumping factor, weak DNase, narrow zone of hemolysis and delayed pigmentation development. Meanwhile, Stephan *et al*. [21] concluded that staphaurex and egg yolk reaction are not suitable for diagnosis of *S. aureus* from milk samples. It has been previously reported that toxic shock syndrome (TSS) secreting strains of *S. aureus* isolated from patients with Kawasaki disease (KD) exhibit an unusual phenotype which distinguishes them from TSS isolates, including the expression of lower levels of hemolysins, lipase and protease [22,23].

It is clear that both human and animal *S. aureus* isolates were positive for the presence of protein A by using agglutination test as shown in Table 5 and Photo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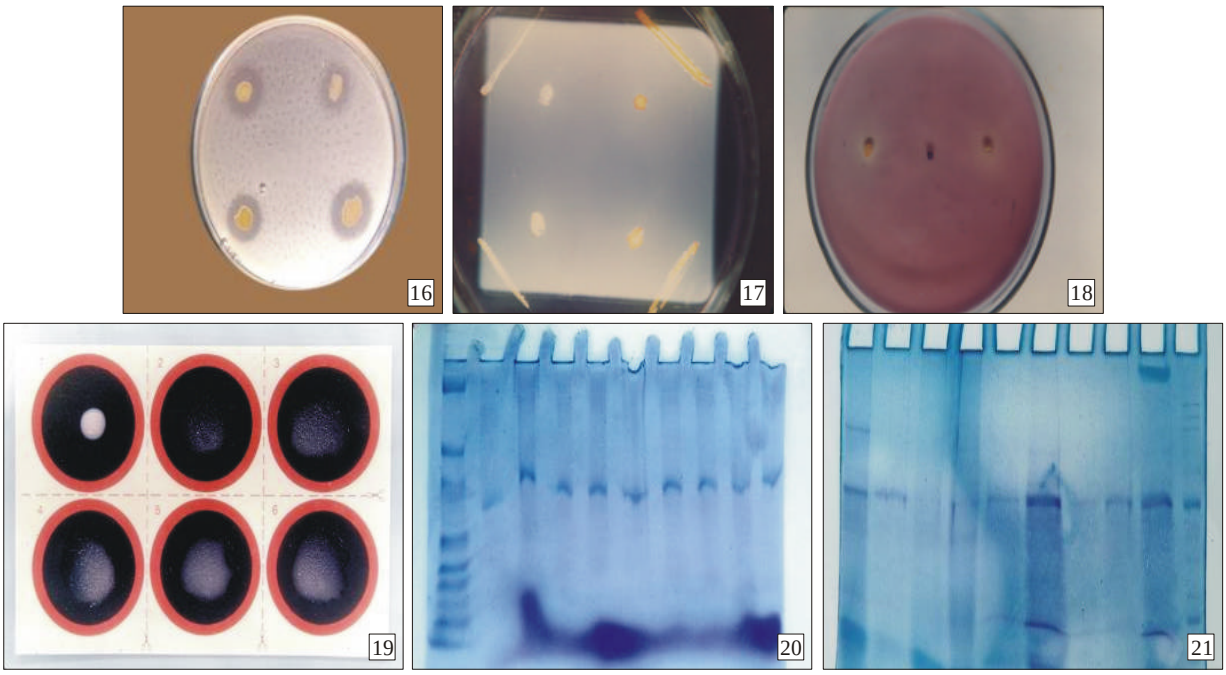

1. Shows coagulase test among the examined *S. aureus* isolates.
2. Shows growth of *S. aureus* on mannitol salt agar medium (yellow).
3. Shows growth of *S. intermedius* on mannitol salt agar medium (red).
4. Shows sensitivity of *S. aureus* to novobiocin disk.
5. Shows resistant of *S. intermedius* to novobiocin disk.
6. Shows hemolytic activity of *S. aureus* on sheep blood agar.
7. Shows DNase positive *S. aureus* isolate.
8. Shows DNase negative *S. aureus* isolate.
9. Shows lysozyme activity of *S. aureus* isolate.
10. Shows gelatinase positive *S. aureus* isolate.
11. Shows tellurite reduction activity of *S. aureus* isolate on Baird-Parker medium.
12. Shows tellurite reduction activity of *S. aureus* isolate on Vogel Johnson medium
13. Shows lipase activity of human *S. aureus* isolate on egg yolk agar medium.
14. Shows lecithinase activity of *S. aureus* isolate on Baird-parker medium.
15. Shows protease activity of *S. aureus* on milk agar medium.
16. Shows fibrinolysin activities of *S. aureus* isolate on human plasma agar medium.
17. Shows growth characters of *S. aureus* isolates on crystal violet medium.
18. Shows growth characters of *S. aureus* isolates on crystal violet medium.
19. SpA activity of *S. aureus* by using agglutination test.
20. SDS profile analysis of 17 *S. aureus* isolates.
21. SDS profile analysis of 17 *S. aureus* isolates.

(19). Previously, Victor *et al*. [24] concluded that, strains of both human and nonhuman animal origin of *S. aureus* were observed to contain protein A except for one strain of biotype D. Kawasaki disease (KD) isolates were found to express significantly more cell wall-associated and extracellular SpA than isolates from patients with toxic shock syndrome (TSS) [4].

Out of 14 human isolates 12 were SpA positive (85.7%), while 33.1% and 25% were positive among *S. aureus* isolated from concentrated ration and chicken samples respectively. The absence of protein A differentiates *S. aureus* of the poultry biotype from *S. aureus* of the *slaughterhouse* biotype as described by Isigidi *et al*. [25]. Protein A was present in all strains

Table 6: Results of chemotherapeutic sensitivity test of 15 *S. aureus* isolates

	Resistant		Intermediate		Sensitive	
Antimicrobial agents	No.	%	No.	%	No.	%
Amoxycillin	10	66.7	1	6.7	4	26.7
Amoxycillin+clavulanic acid	10	66.7	1	6.7	4	26.7
Ampicillian	11	73.3	3	20	1	6.7
Cefoperazone	-	-	1	6.7	14	93.3
Cefotaxime	1	6.7	1	6.7	13	86.7
Ciprofloxacin	2	13.3	3	20	10	66.7
Enroflaxacin	13	86.7	1	6.7	1	6.7
Gentamicin	10	66.7	2	13.33	3	20
Methicillin	2	13.3	1	6.7	12	80
Neomycin	9	60	1	6.7	5	33.3
Ofloxacin	3	20	4	26.7	8	53.3
Oxytetracycline	12	80	1	6.7	2	13.3
Streptomycin	4	26.7	3	20	8	53.3
Sulphamethoxazole/Trimethoprim	2	13.3	2	13.3	11	73.3
Tobramycin	4	26.7	2	13.3	9	60

No. Number

% was calculated according to total number of *S. aureus* examined

isolated from hatchery personnel but not in some strains isolated from broiler parent from personnel [9]. Table 5 and Photos (20 and 21) revealed heterogenecity of a major cell wall protein of the examined human and non human *S. aureus* isolates in the 44.4-46 kDa. Cheung *et al*. [26] reported that the cell wall proteins of 12 *S. aureus* strains revealed heterogeneity of a major cell wall protein in the 45- to 57-likodalton molecular size range on sodium dodecyl sulfate-polyacryl-amide gel electrophoresis (SDS-PAGE) analysis, the cell wall proteins of *S. aureus* provide evidence that SpA variants among serologically distinct strains of *S. aureus* can differ by as much as 12 kilo Daltons in apparent molecular size.

The data here may so far contribute to this assumption as the biochemical characteristics of *S. aureus* originating from different sources did not differ significantly and there is no special test gives the best differentiation for determining the host origin. It can be concluded that one has always be aware of the existence of atypical *S. aureus* strains. In routine bacteriology, these strains can easily be misclassified as non-aureus staphylococci. Since the introduction of antimicrobials into human medicine, staphylococci have shown a frequent and rapid development and spread of antimicrobial resistance, particularly in nosocomial infections. As a consequence, evolution in the frequency of staphylococci resistant to antimicrobial agents has been recorded over the last decades [27,28].

Most of the studies deal with isolates of specific staphylococcal species or staphylococcal isolates of certain animal species obtained in restricted geographic locations and/or clinical situations and therefore, the resulting data are only of limited value for epidemiological considerations. A good example of this is given in the review of Aarestrup and Jensen [29] on penicillin resistance in bovine *S. aureus.* In the present investigation high resistance was recorded to enroflaxacin (86.7%) among the examined *S. aureus* isolates, followed by oxytetracycline (80%) and ampicillin (73.3%), then amoxycillin, amoxycillin clavulanic acid and gentamicin (66.7% each) as shown in Table 6. Meanwhile 93.3% of the examined *S. aureus* isolates were sensitive to cefoperazone, 86.7% to cefotaxime and 80% to methicillin.

It is clear that 13.3% and 6.7% of the examined isolates were resistant and intermediately resistant to methicillin respectively. Methicillin-resistant *S. aureus* (MRSA) was first detected in the 1960s and since that time it has spread rapidly worldwide, becoming a leading cause of nosocomial infections [30]. In human medicine, antimicrobial multiresistance is frequently encountered and methicillin-resistant *S. aureus* strains (MRSA) are among the most threatening bacteria involved in nosocomial infections [1]. Many MRSA infections that appear to have a community onset occur in patients who are found to have had direct or indirect contact with hospitals, care homes or other healthcare facilities [31]. Many other severe cutaneous complications of CA-MRSA (community-associated MRSA) have been reported and include extensive cellulitis, necrotizing fasciitis and purpura fulminans [32]. A recent prospective USA cohort study found that clinical and epidemiological risk factors in persons hospitalized for CA-MRSA infection cannot distinguish reliably between MRSA and MSSA [33]. In veterinary medicine, however, MRSA, as well as multiresistant *S. aureus* strains, are reported only occasionally [34,35]. MRSA isolates detected in animal staphylococci have most been assumed to originate from human sources [29, 36]. In nature, genes on plasmids often encode proteins (e.g., enzymes) that protect the bacterium from one or more antibiotics [37]. As bright as the future looks for new diagnostic tools, prospects concerning new developments of antistaphylococcal drugs for use in animals seem less encouraging.

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