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Antibiotic Resistance Pattern of *Staphylococcus* species Isolated from raw cow milk in Ghebaish Locality

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Abstract:
Staphylococcus aureus is one of the important pathogens involved in food related diseases and common community associated infections. This organism proliferates in food and causes food-borne illnesses. Milk serves as an ideal medium for growth of many microorganisms including *Staphylococcus aureus*. This study was conducted to isolate *Staphylococcus aureus* from raw cow milk and studying its antibiotics susceptibility. Two hundred raw cow milk samples were collected from four areas throughout Ghebaish locality under aseptic precautions and processed under standard bacteriological techniques. The samples were investigated for the presence of *Staphyllococci*. The Baird Parker Agar and Mannitol salt Agar were used as selective media for isolation. The presumptive isolates were identified on the basis of their morphological, cultural and biochemical characteristics. The sensitivity pattern of *S. aureus* with different antimicrobial agents was evaluated by disk diffusion method. Forty five (22%) *Staphyllococci* isolates were obtained and confirmed by biochemical tests, 33 (73%) isolates were *Staphyllococcus aureus*, 12 (26%) isolates were *Staphylococcus epidermis*.The results of sensitivity test revealed high resistance of antibiotics used. The two organisms showed resistant to Streptomycin (0%), Vancomycin (78% - 100%), methicillin (18% - 25%) and penicillin (100%).It is concluded that, *S. aureus* appeared to be a major frequent bacterial contaminant of raw cow milk reflecting potential public health threat. The result of antibiotics sensitivity testing showed variable response and multi-drug resistance.

Key words: Antibiotic, resistance, *Staphyllococci*, milk**.**

Introduction:

Milk is a highly valuable food, but raw milk contains and favors growth of many microorganisms, milk is a completely balanced diet with the right amount of carbohydrate, protein, fats, vitamins and minerals. Bacteria of sorts thrive in milk and as a result reduce its quality. The presence of pathogenic bacteria in milk is of immense public health significance. The hands of unhygienic milk handlers, the housing environments and instruments, and the cow itself are possible sources of milk contamination by pathogenic bacteria (Capurro *et al.,* 2010).

Staphylococcus aureus which is one of the causes of mastitis in cows could have its source from milk handlers since most humans carry the organisms in their nostrils. This is the most common type of mastitis and has great economic importance to dairy farmers (Abera *et al*., 2010).

Mastitis in cattle caused by *S. aureus* can either be subclinical or clinical. According to Bachaya *et al*. (2011), sub-clinical mastitis is of global importance in the dairy industry. It

shows no noticeable alterations in the appearance of the milk or the udder, but there is decrease in milk production. The symptoms of clinical mastitis include swelling, hardness, redness, heat, and pain.

It is very important to implement application of an antibiotic susceptibility test prior to the use of antibiotics in both treatment and prevention of intra-mammary infections. This is because the presence of *S. aureus* or any pathogenic bacteria in milk can be a transmission pathway to humans. It becomes more dangerous when the pathogen develops resistance to antibacterial agents. Antibiotics resistance has become a very important public health issue globally (Abebe *et al*., 2013).

The presence of *Staphylococcus aureus* in raw milk generally comes from cows with mastitis, from handlers or from deficient hygiene. When found in milk, high levels of contamination can be reached quickly under favorable conditions. Its presence in foods can be a risk to human health, causing a public health problem, as these bacteria produces toxins that can cause toxic food infections (Quintana and Carneiro , 2006)

The capacity to coagulate plasma, the principal characteristic of the *Staphylococcus aureus*, is highly correlated to the capacity to produce enterotoxins harmful to the tissues of the contaminated host (Murray *et al.*, 2006).

Milk can be contaminated by *Staphylococcus aureus* when there is infection of the mammary gland or by bad hygiene habits, such as coughing or sneezing and not washing hands when handling milk storage equipment, during or after milking, and in this case, human activity is responsible for the contamination, as this bacteria colonizes the nasal pathways in human beings (Fagundes and Oliveira, 2004).

Nowadays, about 70 percent of the bacteria that cause infections in hospitals are resistant to at least one of the drugs most commonly used for treatment. Some organisms are resistant to all approved antibiotics and can only be treated with experimental and potentially toxic drugs. An alarming increase in resistance of bacteria that cause community acquired infections has also been documented, especially in the *Streptococcus pneumonia* which is prevalent cause of disease and mortality. In a recent study, 25% of bacterial pneumonia cases were shown to be resistant to penicillin, and an additional 25% of cases were resistant to more than one antibiotic (Rhee and Gardiner, 2004).The resistance is due to wrong antibiotic usage or irrational use of drugs. Bacteria may acquire drug resistance due to chromosomal mutation, transformation, conjugation or transduction. Antibiotic-resistance likely also emerged as bacteria began producing compounds in order to survive in their environment, and competing species found ways to counteract these compounds, (Matthew, 2007).

Thus, the objective of this study was to isolate *Staphylococcus aureus* from raw cow milk in Ghebaish Locality and evaluation of the drug resistance pattern of *S. aureus* isolates using different antibiotic agents.

Materials and Methods:

Collection of samples

A total of 200 raw cow milk samples were collected from four sectors that lies at Ghebaish Locality between October and December 2017, including Eastern sector, Western sector, Almansoura sector and Aljaflah sector. The samples were collected in sterile universal bottles and immediately taken to the research laboratory of College of Veterinary Medicine in West Kurdufan University for culturing and bacteriological investigation for the presence of *Staphylococcus* species.

The isolation and Identification of *Staphylococci*

This was done according to (Cheesbrough, 2000; Barrow and Feltham, 2003; Collins, *et al.,* 2004). Mannitol salt agar and Baird parker's agar as selective media (Oxoid,2006). All primary tests were applied including were used enzymes production (oxidase and catalase)

and gram stain. Secondary tests were included sugars fermentation (glucose, sucrose, trehalose, mannitol and maltose), urea utilization, coagulase, blood heamolysis Vogues proskauer test Analytical Profile Index (API Staph) was used for further confirmation (Layer, 2006).

Sensitivity test

Staphyllococci isolates were cultured in Muller Hinton agar and incubated at 37C°for 24 hours. According to CLSI (2007), following antibiotics: Streptomycin, Vancomycin, Methicillin and Penicillin were used.

Results

The *Staphylococcus* isolates obtained from raw cow milk in Ghebaish Locality are shown in (table 1).The number of isolates were 45(22%) and the organisms isolated were *S.aureus* (33 / 73%) and *S. epidermities* (12 / 26%).

Table (1) *Staphyllococcus spp* isolated from cow milk different sectors in Ghebaish Locality.

Area	*S.aureus*	*S.epidermities*
Eastern sector	18 (54 %)	4 (33%)
Western sector	5 (15%)	3 (25%)
Almansoura sector	10 (30%)	5 (41%)
Aljaflah sector	0 (0%)	0 (0%)

Results of Antimicrobial susceptibility test

Staphylococcus isolates were exposed to four antibiotics; the response varied with each species.

Table (2) Resistance of *Staphylococcus* isolates to four antibiotics in terms of percentage.

Antibiotic	Sterptomycin	Vancomycin	Methicillin	Penicillin
Total *S.aurus* (R)	0 (0%)	26 (78%)	6 (18%)	33 (100%)
Total *S.aurus* (S)	31(93%)	7 (21%)	3 (9%)	0 (0%)
Total *S.aurus* (I)	2 (6%)	0 (0%)	24 (72%)	0 (0%)
Total *S.epidermities* (R)	0 (0%)	12(100%)	3 (25%)	12 (100%)
Total *S.epidermities* (S)	12 (100%)	0 (0%)	0 (0%)	0 (0%)
Total *S.epidermities* (I)	0 (0%)	0 (0%)	9 (75%)	0 (0%)

*R = Resistance, * S = Sensitive, *I = Intermediate Resistance

Total = Total number of isolates interacts with antibiotic.

Discussion:

Although *Staphylococci* are normal flora of skin and mucous membranes of man and animal body, some of them have been associated with many health problems. They might cause disease by their presence in the animal body or by contaminating the food. Staphylococcal infections cause significant morbidity and mortality in both the community and hospital setting (Cosgrove *et al*., 2003).Milk is normally sterile in the udder of the cow and buffalo provided they do not suffer from mastitis (udder infection). If they have mastitis, a large number of generally Gram positive bacteria such as *Streptococcus* and *Staphylococcus* spp. may be present in milk when it leaves the udder (Holm and Jespersen, 2003).The results obtained from this research contamid the occurrence rate of subclinical mastitis among the 200 cows was 22% (45 cows). This report is lower than the report of Sharma and Brinty

(2014) with a prevalence of 30.9%in subclinical mastitis and that of Ameh *et al*. (1999) with a prevalence of 31% from settled herds in Zaria and 57.7% in cows in Maiduguri by Bamayi and Aniesona (2013). The presence of *Staphylococcus aureus* also reflects. Deficient sanitary conditions of the cattle herd given that *Staphylococcus aureus* is predominant as the cause of most cases of mastitis.Many coagulase-negative *Staphylococci* were isolated in this study. Recently, the coagulase negative staphylococci have been studied extensively because of their pathogeincity and involvement in some kinds of human and animal diseases (Pfaller and Herwaldt, 1988; Kloos and Bannerman 1994; Buttery *et al*., 1997; Mahoudeau *et al*., 1997).Drug resistance now a day become a problem, because many types of bacteria exhibit resistance to many types of antibiotics used to treat diseases associated with animal and human health. Prescription of antibiotics in Sudan depends mainly on clinical diagnosis and sometimes without veterinarian supervision. In this study all isolates showed resistance to penicillin, this can be explained by the fact that penicillin was the most frequently used antibiotic in Sudan in many applications. Another study in Bangladesh conducted by Begum *et al*. (2007) revealed that *S. aureus* was 82.86% resistant to Penicillin, however in this study; the antibiotic showed 100% resistant by *S. aureus* and *S.epidermities*, indicating increasing resistance of the organism against Penicillin. Similar types of resistance pattern also reported by Islam *et al*. (2007).

All isolates of *S. epidermities* were resist to vancomycin while only 26 (78%) isolates of *S.aureus* resist it, which is higher than (13%) recorded by Ahmed (2004).

The resistance of *S. aureus* to methicillin was found to be 18%. Usually methicillin is not widely used in livestock treatment, for that reason may be; some isolates were found to be at intermediate resistance to methicillin. Also (25%) *S. epidermities* isolates was resistant to methicillin the isolates were fully sensitive to streptomycin in contrast to (57%) resistance obtained by Anueyiagu and Isiyaku (2015). The antibiotic sensitivity pattern of the isolates were significantly interesting and alarming for livestock and public health sector in Ghebaish locality.

Conclusion:

The study showed that *Staphylococcus* spp. in general, and *S. aureus* in particular, are common in raw cow milk in the study area and may impose a public health hazard. Different *Staphylococcus sp* shows various responses to antimicrobial agents, All *Staphylococcus* isolates were sensitive to streptomycin and resistant to penicillin. Some isolates resist vancomycin and methicillin with different rates**.**

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