

Survey of Antibiotic Resistant *Staphylococcus species* from fresh Meat samples in Ghebaish City - West Kordofan State – Sudan

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ABSTRACT:

Staphylococcus aureus is an important pathogen that can cause Staphylococcal Food Poisoning (SFP), meat and meat products are frequently contaminated by this bacterium. Multidrug-resistant Staphylococci has become a major public health concern in Sudan and many countries, causing failure in treatment with consequent huge health burden. The isolation, identification of *Staphylococcus sp* from fresh meat and its antibiotic susceptibility was studied. Eighty fresh meat samples from a Slaughter houses and butcheries at Ghebaish city were randomly collected for this study, and samples were collected during the period 15/3/2019 – 9/4/2019. All samples were transported to the laboratory under aseptic conditions for further investigation. A total of thirty two coagulase positive *Staphylococcus sp* isolates was obtained from all samples based on their colonial morphology and biochemical characteristics. In this study, the sensitivity of thirty two isolates to thirteen antibacterial agents was examined. Very high resistance rates to antimicrobial agents were recorded. *Staphylococcus sp.* isolates were fully resistant to Ampicillin, Cefotaxime, Cefuroxime, Amoxicillin, Doxycycline, Vancomycin, Kanamycin and Clindamycin (100%), other antibiotics including Erythromycin (37.5%), Chloramphenicol (25%), Tetracycline (6.25%), Co-trimoxazole (12.5%) and Amikacin (81.25%). The study concluded that *Staphylococcus sp* appeared to be a major frequent bacterial contaminant of meat from animal as well as subsequent contamination of meat during the process of mincing and handling, multiple resistances to antimicrobial drugs among Staphylococci isolates complicate therapeutic management of infections. and it is recommended that over use and misuse of antibiotics is to be prevented as well as raising of hygienic public awareness because of the fact that food hygienic depend on the hygienic and awareness food worker's. There for we advise to raise hygienic awareness food worker's.

Keywords: Multi-drug resistance, Staphylococci, Ghebaish.

Introduction:

Meat is a major constituent of the human diet in Sudan. It is an essential food item and one of the main sources of protein, fats, minerals and vitamins. Most meat have high water content corresponding to the water activity approximately 0.99 which is suitable for microbial growth (Rao *et al.*, 2009). Meat is considered as an important source of proteins, essential amino acids, B complex vitamins and minerals. Due to this rich composition, it offers a highly favorable environment for the growth of pathogenic bacteria Gill (1998). Meat products are perishable and unless processed, packaged, distributed and stored appropriately can spoil in relatively short time Sofos, (2005).

Meat is subjected to changes by its own enzyme, by microbial action and its fat may be oxidized chemically microorganisms grow on meat causing visual, textural and organoleptic change when they release metabolites (Jackson *et al.*, 2001). Meat is a good material for bacterial growth; its quality depends on the initial bacterial contamination. This contamination causes meat deterioration, lowers quality and sometimes illness may be caused by bacterial pathogens or their toxins through meat and meat products. Generally, animal proteins such as meats, meat products, fish and fishery products are generally regarded as high risk commodity in respect of pathogen contents, natural toxins and other possible contaminants and adulterants (Yousuf *et al.*, 2008). In fact, tissue from healthy animal are sterile however, it has been pointed that during slaughter, dressing and cutting, microorganisms came chiefly from the exterior of the animal and its intestinal tract but that more added from knives, cloths, air, carts and equipment in general. External contamination of meat is a constant possibility from the moment of bleeding unit consumption (Lawrie, 1984). Among the factors that affect microbial growth in meat are intrinsic properties (physical and chemical properties of meat) and extrinsic (environmental factors) (Rombout and Nout, 1994), however the factors having

the greatest influence on the growth of microorganisms in meat and meat products are the storage temperatures, moisture and oxygen availability (Forest *et al.*, 1985). The possible sources of these bacteria are likely to come from the skin of the animal from which the meat was obtained. Other potential sources of microbial contaminations are the equipment used for each operation that is performed until the final product is eaten, the clothing and hands of personnel and the physical facilities themselves are all implicated (Rombouts and Nouts, 1994). Retail cut could also result in greater microbial load because of the large amount of exposed surface area (Forest *et al.*, 1985).

Many bacterial pathogens were reported as meat contaminants. CDC (2000) reported that, pathogenic microorganisms that were involved in outbreaks associated with consumption of meat and meat products during the period 1993-1997 were *Bacillus cereus*, *Campylobacter*, *Clostridium botulinum*, *Clostridium perfringens*, enteropathogenic *E. coli*, *Salmonella*, *Shigella*, *Staphylococcus aureus* and *Yersinia enterocolitica*.

However in Sudan; there are studies on the genera of aerobic bacteria in fresh meat *Bacillus* spp., *Micrococcus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Acinetobacter* spp. and *Proteus* spp. were isolated from beef. But additional research is needed to know the species of each specific genus (Hussein, 1987, Mohammed, 2000).

Staphylococcus aureus is representing the most frequent zoonotic food-borne pathogen isolated from food of animal origin that requires understanding its molecular ecology in food, especially raw meat harboring isolates containing multiple toxin genes (Song *et al.*, 2015).

S. aureus can produce several virulence factors including enterotoxins which are heat stable, retain their biological activity even after thermal processing of food and also resistant to gastrointestinal proteases such as pepsin, also, it remains one of the most intensively investigated bacterial species in human and animals. It is an adaptable, opportunistic pathogen with abilities to persist and multiply in a variety of environments and causes a wide scale of diseases (Cremonesi *et al.*, 2007).

Antimicrobial resistance is a main public health worry worldwide. Antibiotic resistance is defined as the ability of a micro-organism to grow or survive in the presence of an antibiotic that is usually sufficient to inhibit or kill microorganisms of the same species, the expansion of resistance both in humans' and animals' bacterial pathogens has been allied with the widespread remedial use of antimicrobials or their administration to food producing animals as growth promoters (Barber *et al.*, 2003). *S. aureus* has become resistant to various antimicrobial agents including the commonly used penicillin-related antibiotics as oxacillin, methicillin and other beta lactams (Boyce *et al.*, 2005).

According to the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC), multi-drug resistant (MDR) is defined as non-susceptibility to at least one agent in three or more antimicrobial categories. MDR bacteria are the principal cause of failure in the treatment of infectious diseases, resulting in increases in the term and magnitude of morbidity, higher rates of mortality, and a greater health cost burden. However, recently researchers have been investigated the activity of non-antimicrobial agents such as proanthocyanidin to prevent MDR bacterial infections (Gupta *et al.*, 2012).

The present study was designed to investigate the incidence of antibiotic resistant *Staphylococcus species* from fresh meat samples at Ghebaish city.

Materials and Methods:

A total of 80 samples were taken randomly from a Slaughter houses and butcheries at Ghebaish city under a septic condition and stored in sterile clean universal bottles. The samples were immediately transported on ice in container with sufficient speed to avoid unnecessary delay and/ or contamination prior to microbiological examination. The culturing and biochemical analysis and bacteriological investigation were done at Microbiology Research Laboratory of the College of Veterinary Medicine, West Kordofan University...

The samples were streaked on Mannitol Salt Agar (MSA) and Blood Agar (BA) plates. The plates were all incubated at 37°C for 24 hours, after which the cultural and morphological characteristics of the isolates were studied. Identification of isolates was by standard microbiological methods as described by Cheesbrough (2000) and Cowan

(2003), significant bacterial growth was included in this study and they were identified on the basis of cultural characteristics, gram stain and conventional biochemical tests like catalase, oxidase, coagulase, O/F, VP, MR and sugar fermentation.

The antimicrobial sensitivity testing was carried out by disc agar diffusion technique (Dechet *et al*, 2006). Using Muller-Hinton agar plates. The disc used contained the following antibiotics: Ampicillin, Erythromycin, Chloramphenicol, Tetracycline, Cotrimoxazole, Cefotaxime, Cefuroxime, Amoxicillin, Amikacin, Doxycycline, Vancomycin, Kanamycin and Clindamycin. Isolates were divided into three groups based on the zone of inhibition produced by the antibiotic disc; susceptible, intermediately susceptible and resistant according to the Clinical and Laboratory Standards Institute (CLSI) guideline; Performance Standards for Antimicrobial Susceptibility Testing (CLSI, 2007).

Results:

A total of 32 (40%) *Staphylococcus sp* (able to grow on mannitol salt agar) were isolated from 80 samples subjected to bacteriological examinations. They are positive in the catalase and coagulase tests.

The organisms exhibit remarkable versatility in their behavior towards antibiotics as shown in figure (1):

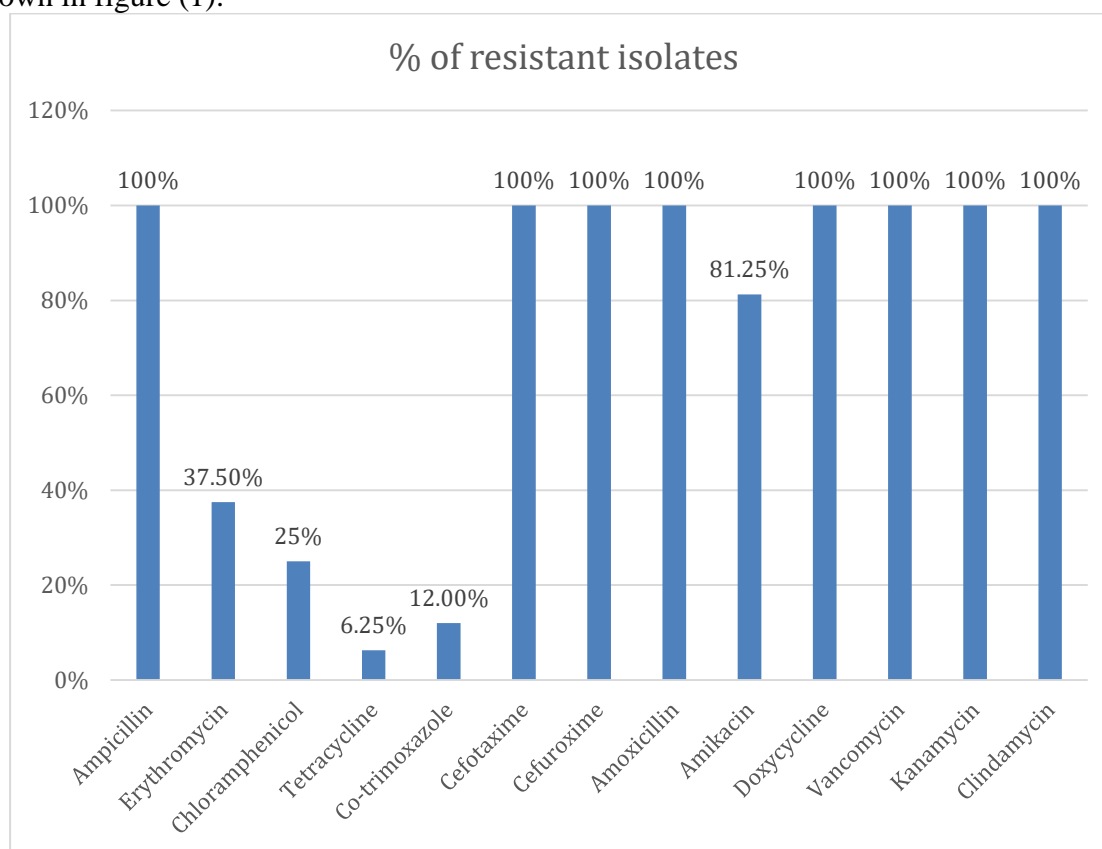


Figure:

(1) Antibacterial resistance patterns of *Staphylococcus species* against used antibiotics.

Discussion:

In this present study, different fresh meat samples were collected for the isolation of *Staphylococcus sp*. This was done to study the properties of one of the most important pathogens in different samples of this microorganism responsible for economic loss and public health problems.

Food handlers are usually the main source of food contamination. However, the surfaces of equipment and machines of mincing meat can also be a source of Staphylococcal contamination

In this study many *Staphylococcus* isolates were obtained from fresh meat they could be originated from food animal or other environmental sources. The results revealed that 40% of total samples collected was contaminated with *Staphylococcus sp* and may cause several illnesses.

The presence of these organisms on meat could be attributed to the fact that meat contains an abundance of all nutrients required for the growth of bacteria in adequate quantity. The contamination with these organisms in this study may be due to condition of the handling, tables, equipment and machines, which had been used during processing, preparing, and the hygienic practice, employed by butchers.

This study agrees with Bhandare *et al.* (2008), due to improper/unhygienic handling and processing of the meat, meat is normally transported to the markets in unhygienic meat vans, taxis, motor cycles, motor kings, and sometimes on donkeys. It is also a common practice to see people carrying carcasses on their bare shoulders. According to Bhandare *et al.* (2008), the unhygienic practices of meat processing in developing countries results in these meat been contaminated with microorganisms. Meat sellers were also observed busily conversing, coughing, and sneezing, which might result in contamination through introduction of saliva. Okonko *et al.* (2008) stated that, food can be infected with microorganisms as a result of “coughing” and “sneezing” from those who handle and process these foods.

Another study by Jay (2005) revealed that the workers hands and the equipment were the sources of meat contamination and these results are in accord with the present results.

The present study investigated the prevalence of *Staphylococcus sp* in fresh meat samples randomly collected in Ghebaish city. The results of 80 fresh meat samples showed a high recovery rate of coagulase positive *Staphylococcus sp* (40%).

Results of the sensitivity tests showed variety levels of Staphylococci response to different antibiotics and this may be due to antibiotics misuse.

The antibiotics to which Staphylococci showed full resistance percentage (100%) were Ampicillin, Cefotaxime, Cefuroxime, Amoxicillin, Amikacin, Doxycycline, Vancomycin, Kanamycin and Clindamycin and this may be attributed to in discriminate antibiotics usage.

Most of the microbial resistance which is now making it difficult to treat some infectious diseases is of genetic origin and transferable between species and genera of bacteria. It's due to extensive use of antimicrobial drugs which have favored the emergences of resistant strains. The overuse and misuse of antimicrobials have lead to the death of sensitive strains leaving resistant strains to survive multiply and infect new hosts.

In this study all isolates showed full resistance to ampicillin. This can be explained by the fact that ampicillin was the most frequently used antibiotics in Sudan generally. Hence, bacteria are more likely to develop resistance against them. Actually, the medical authority inhibits the use of ampicillin for curing bacterial infections in the State due to the increased bacterial resistance.

Some of Staphylococci isolates showed resistance to tetracycline (6.25%), this is a highly resistant isolates which can indicate a pathogenic strain. This result was in agreement with that who reported by El Sanosi (2005) that confers tetracycline resistance, was particularly common, and was identified in Staphylococci. Tetracycline is widely used in the Sudan in many applications, namely for topical treatment of wounds (as powder), as premeditation in poultry (prophylaxis), and as injection for veterinary use (oxytetracycline).

The wide spread use of antibiotic therapy, especially in hospitals, has led to the spread of R-plasmids among pathogenic bacteria. These might explain the high multi-resistance observed in this study.

It was suggested from the finding of this study that staphylococci isolated from minced meat may contains antibiotic resistance genes in the bacterial chromosome and/or plasmids.

Antibiotic multi-resistance of some bacteria may be due to the resistance plasmids (R-plasmids), which contain genes that render the host bacterium resistant to sulphonamides, streptomycin, chloramphenicol, kanamycin and tetracycline. Some R-plasmids confer resistance to up to eight different antibiotics (Carter, 1985; Schlegel, 1995). This property of several antibiotics resistance could be transferred from one bacterium to another by simple cell-to-cell contact during conjugation (Carter, 1985; Schlegel, 1995). Some R-plasmids have an extensive host range and can transfer between several genera (Carter, 1985; Schlegel, 1995). This could explain the wide distribution of multi-antibiotic resistance observed in this study. Also transposon (Tn element) which sometimes code for resistance to antibiotics such as penicillin, tetracycline and kanamycin etc, able to

move between bacterial chromosomes and plasmids (Schlegel, 1995) and might attributed to high multi-antibiotic resistance observed in this investigation.

Through the process of spontaneous mutation and selection by misuse and overuse of antibiotics, some bacteria develop inheritable multi-drug resistance. This may be one of the factors contributed in multi-resistance observed in bacterial isolate of this study.

Conclusion:

The study concluded that The general sanitary conditions at the meat shops in addition to poor hygienic practices by the butcher are probable contributors to the microbial contamination on the meat especially *Staphylococcus sp* and the result of antibiotics sensitivity testing showed response and resistance of the different isolates to different antibiotics were varied associated with resistance to more than one antimicrobial agent (MDR Multi Drug Resistance).

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