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Pankaj Mangal
Former Project Associate, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Munesh Kumar Pushp
Teaching Associate, Pashu Vigyan Kendra, Kumher, RAJUVAS, Bikaner, Rajasthan, India

Tushar Maharishi
Ph.D. Scholar, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Ashok Prajapat
Assistant Professor, Arawali Veterinary College, Sikar, Rajasthan, India

Corresponding Author:
Pankaj Mangal
Former Project Associate, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Assessment of *Staphylococcus aureus* in raw pork from retail meat outlets of Bikaner: Isolation, identification and antibiotic sensitivity pattern

Pankaj Mangal, Munesh Kumar Pushp, Tushar Maharishi and Ashok Prajapat

Abstract
Abundance pathogenic bacteria, including non-typhoid Salmonella serovars, *Escherichia coli*, and *Staphylococcus aureus*, are found in pork and represent a risk to human health as well as potential sources of infection. Pork can transfer a large variety of microorganisms to humans, although few of them are expected to have a significant effect on public health. Fifty raw pork samples were collected from different retail meat outlets in Bikaner city for this investigation, and they were evaluated for isolation, identification and antibiotic sensitivity test of *Staphylococcus aureus*. Thirty six of the 50 raw pork samples were positive (72.0%) for *Staphylococcus aureus*. 13 (41.67%) of the thirty-six *Staphylococcus aureus* isolates from pig samples were coagulase positive, while 21 (58.33%) were coagulase negative. All 36 *Staphylococcus aureus* isolates were subjected to antibiotics sensitivity test with eleven various types of antibiotics. Isolates of *Staphylococcus aureus* exhibited great resistance to Penicillin-G and vancomycin and maximal sensitivity to ciprofloxacin and sulpha triad.

Keywords: Pork, standard plate count, coagulase, sulphatriad, vancomycin

Introduction
Pigs were first domesticated approximately 5000 years ago. Because of their high feed conversion ratio, high prolific rate, short gestation period, excellent climatic and food adaptation, pigs have a lot of potential to meet the increasing worldwide demand for meat (Pond *et al* 1991) [3]. Word ‘pork has been derived from the French 'porc' and Latin 'porcus' meaning "pig". It has been demonstrated that pork is a valuable food source globally, contributing to roughly 40% of all meat produced (Sherikar *et al* 2013) [33].

Pork has a high nutritional value and is an excellent source of important nutrients. It also tastes delicious and is healthful. But being nutritious also makes this food highly perishable and also a potent source for food borne infections. One excellent method of getting pathogenic microbes into the larger population is through retail pork. Since antibiotic resistance genes can transferred within non-pathogenic bacteria and pathogenic or opportunist bacteria in the intestine, food contamination by antibiotic-resistant bacteria may result in a serious risk to public health. Dealing with a severe bacterial infection could become more difficult as a result (Sorum and L’Abee, 2002; Swartz, 2002) [35, 36].

A significant cause of food-borne disease has been associated with pork (EFSA, 2008) [10]. Unhygienic production of pork, openly displayed pork products in which predispose transmission of bacterial diseases such as Salmonella, Yersinia, Staphylococcus, and *Escherichia coli* to the consuming public is made possible by contact between consumers and insects. It has been demonstrated that handling raw materials is critical for meat safety (Mormur and Yuste, 2010) [25].

Staphylococcus aureus is another organism of concern as it has the ability to create a wide range of extracellular protein toxins, including coagulase, hemolysins, exfoliative toxin (ET), toxic shock syndrome toxin 1 (TSST-1), and at least 15 different forms of enterotoxins (Mehrotra *et al* 2000 and Normanno *et al* 2005) [23, 27].

Antimicrobial resistance or sensitivity in bacteria has been considered an important health problem in recent decades. Because of the frequent misuse and overuse of antibiotics (in livestock husbandry, agriculture, and the treatment of human illnesses), the proportion of bacteria resistant to antimicrobial drugs is rapidly increasing. Due to a widespread consumption of antibiotics, isolates from food have shown a significant increase in resistance against most antibiotics over the past ten years (Valsangiacomo *et al.*, 2000) [38].

In developing countries, the consumption of food derived from animals has been steadily increasing. The main causes of this increasing consumption of animal-based food are population growth, urbanization, and increasing wealth. One of the first animal foods is pork. Even so, India's consumption of it has not been very high due to religious believes but data from 2009 and 2010 indicate that the production of pork increased by 0.18% in Rajasthan alone, indicating a gradual increase in its acceptance as food (FAO, 2012) [13].

The population's pork consumption habits have been influenced by geographic, religious, and unique cultural traditions, yet it is consumed in various parts of world (Kumaresan *et al.*, 2009) [20]. Foods of porcine origin are considered to be one of the sources for *E. coli* and *Staphylococcus aureus* illnesses in humans (Dias *et al.*, 2013) [9]. These microorganisms found in cooked pork illustrates the appalling conditions of the inadequate hygienic and sanitary procedures used in the processing, packaging, and slaughtering of pork (Yannick *et al.*, 2013) [40].

Materials and Methods

The present research was carried out at the Department of Veterinary Public Health, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, India.

For this study, 50 pork samples total were collected from different retail meat outlets in Bikaner city. Ten to twenty grams of pork samples were collected in sterile test tubes and brought straight to the laboratory in cold conditions. The samples were processed in four to six hours after collection.

The pour plate method, as outlined by Banwort (1989) [4], was used to perform the Standard Plate Count (SPC) on pig samples. In order to obtain isolated colonies of bacteria, each pig sample was streaked in primary, secondary, and tertiary form on Nutrient agar and MacConkey (MCA) agar plates. These petri plates were incubated at 37 °C for 24 hr. For the purpose of isolating *Staphylococcus aureus*, these isolated colonies were cultivated on Mannitol Salt Agar (MSA) plates and incubated for a 24 hr at 37 °C. Different types of colonies were sub-cultured on different nutrient agar plates in order to produce pure culture, and the growth was evaluated for colonial morphology and pigmentation.

The isolates' confirmation as *Staphylococcus aureus was* confirmed by Gram staining, the Hi StaphTM Identification Kit (HiMedia, Mumbai) which included a set of 12 biochemical assays, as well as the Catalase, Oxidase, Coagulase, and oxidase tests. The Coagulase test was carried out using the Cowan and Steel (1975) [7] method. The protocol recommended by Kirby *et al.* (1966) [18] was followed while conducting the antibiotic sensitivity test (ABST). Eleven antibiotic discs Ampicillin, Chloramphenicol, Ciprofloxacin, Erythromycin, Gentamicin, Lincomycin, Penicillin-G, Streptomycin, Sulphatriad, Tetracycline, and Vancomycin were selected in order to perform an antibiotic sensitivity test.

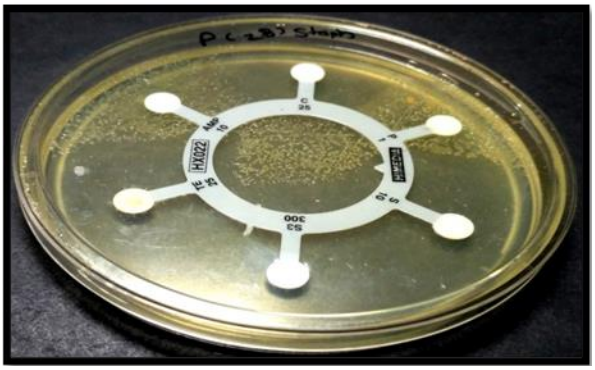

Fig 1: Antibiotic sensitivity test conducted on *Staphylococcus aureus* isolates

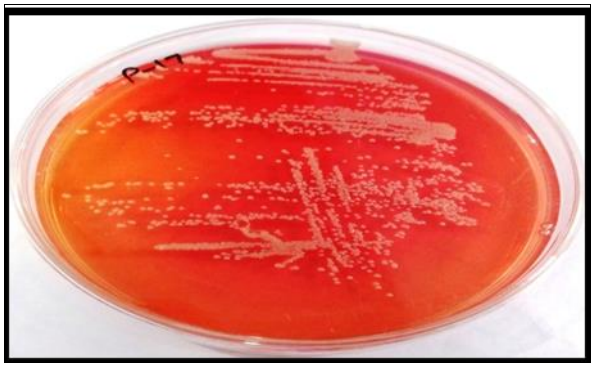

Fig 2: Isolation of *Staphylococcus aureus* on Mannitol salt agar from pork samples

Fig 3: Results of biochemical tests for *Staphylococcus aureus* obtained from Hi*Staph*TM commercial kits

Results and Discussion

In the current study, 36 (72.0%) of the 50 raw pork samples tested positive for *Staphylococcus aureus*. Twenty-one (58.33%) of the 36 *Staphylococcus aureus* isolates from pork samples did not exhibit coagulation and were considered to be coagulase negative. Fifteen (41.67%) were determined to be coagulase positive. *Staphylococcus aureus* was isolated by Daniyan (2011) [8] from samples of chopped and fresh pork. *Staphylococcus aureus* was found in pork sample (Bradeeba and Sivakumaar's 2013) [6]. *Staphylococcus aureus* was the most frequently noticed bacteria in the pork samples (Anachinaba *et al*. 2015) [1].

Staphylococcus aureus was detected in 56.4% of the pork samples (Onpirom *et al*. 2005) [29]. In pig samples, 69% of *Staphylococcus aureus* was isolated (Angkititrakul *et al*. 2013) [2]. Pork samples revealed 81.8% *Staphyloccocus aureus* (Yannick *et al*. 2013) [40]. *Staphyloccocus aureus* was detected in 28.75% of the pork samples reported positive, according to Ashraf *et al*. (2015) [3]. *Staphylococcus aureus* bacteria were found in 29 (36.3%) of the pork samples (Shimizu and Horie, 1999) [34]. 100% of the samples tested

positive for *Staphylococcus aureus*, according to Kumar (2005) [19]. 10.7% of pork samples tested positive for *Staphylococcus aureus* (Boer *et al*. 2009) [5]. In contrast, 45.6% of pork samples were confirmed by Pu *et al*. (2009) [32] to have the bacteria, while 19.0% of pork samples were isolated by Kyung *et al*. (2010) [21] to have the bacteria. Jaisue and Angkititrakul (2011) [15] isolated *Staphylococcus aureus* from 26.06% of pork samples. Hanson *et al.* (2011) [14] reported the prevalence rate of *Staphylococcus aureus* from fresh raw pork samples to be 18.2 %. O'Brien *et al.* (2012) [28] isolated *Staphylococcus aureus* from 67.3% of conventional pork samples and from 56.8% of alternative pork samples. Fahrion *et al*. (2014) [11] also observed 47.6% of the pork samples had *Staphylococcus aureus*. Nnachi *et al*. (2014) [26] also isolated MRSA from 85.7% of pork samples. While, Mathenge *et al*. (2015) [22] observed 37.4% pork samples positive for *Staphylococcus aureus*.

Staphylococcus aureus was confirmed to be present in all of the Gram-positive cocci grown on Mannitol Salt Agar in this study. On Mannitol Salt Agar, *Staphylococcus aureus* developed glistening, smooth, spherical colonies with varying degrees of pigmentation (golden yellow). They were also catalase positive and oxidase negative. The pigmentation grew with the passage of time These isolates did not show growth on Mac Conkey agar. On HiStaph™ commercial kits *Staphylococcus aureus* showed positive reaction for the Voges proskauer's test, Alkaline phosphatase test, Urease test, Arginine utilisation test, and Mannitol, Sucrose, Lactose, Trehalose and Maltose sugar tests while with ONPG, Arabinose and Raffinose test it showed negative reaction.

Comprehensive findings from multiple biochemical assays conducted on *Staphylococcus aureus* are shown in table-1.

Table 1: Results of *Staphylococcus aureus* biochemical analysis obtained from commercial kits HiStaph TM

S. No.	Test	Positive		Negative	
		Number	%	Number	%
01.	Voges roskauer's	31/36	86.11%	5/36	13.89%
02.	Alkaline phosphatase	36/36	100.00%	-	-
03.	ONPG	4/36	11.11%	32/36	88.89%
04.	Urease	36/36	100.00%	-	-
05.	Arginine utilisation	36/36	100.00%	-	-
06	Mannitol	36/36	100.00%	-	-
07	Sucrose	33/36	91.67%	3/36	08.33%
08.	Lactose	36/36	100.00%	-	-
09.	Arabinose	3/36	08.33%	33/36	91.67%
10.	Raffinose	4/36	11.11%	32/36	88.89%
11.	Trehalose	36/36	100.00%	-	-
12.	Maltose	36/36	100.00%	-	-

Eleven distinct antibiotic groups, including ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, lincomycin, penicillin-G, streptomycin, sulphatriad, telcycline, and vancomycin, were used in antibiotic sensitivity tests against each of the 36 *Staphylococcus aureus* isolates. Three categories were established for responses of organisms to antibiotics: sensitive, intermediate, and resistant.

The profile of antibiotic resistance exhibited by the *Staphylococcus aureus* isolates tested included the following: 36 isolates exhibited 100% sensitivity to ciprofloxacin and sulphatriad; 31 isolates demonstrated 86.1% sensitivity to chloramphenicol; 28 isolates demonstrated 77.7% sensitivity to tetracycline; 24 isolates demonstrated 66.7% sensitivity to gentamicin; and 22 isolates demonstrated 61.1% sensitivity to streptomycin.

Additionally, it was reported in the current study that all 36 isolates had 100% resistance to penicillin-G, 32 isolates had 88.9% resistance to vancomycin, 30 isolates had 83.3% resistance to ampicillin, 26 isolates had 72.2% resistance to lincomycin, and 21 isolates had 58.3% resistance to erythromycin.

The exact agreement of the isolates' 100% resistance to penicillin-G to the findings of Thiep *et al* (2014) [37]. A resistance of 96.3% for penicillin-G was also reported by Molla *et al* (2011) [24]. There have been reports of 68% and 66.9% antibiotic resistance to ampicillin (Pu *et al*, 2011; Fan *et al* 2015) [31, 12]. Karpiskova *et al* (2009) [16] reported the antimicrobial resistance profile in some MRSA isolates and observed 70.6% resistance of these MRSA isolates to erythromycin. Almost similar findings for antimicrobial resistance to erythromycin were also reported by Molla *et al* (2011) [24]. Jaisue and Angkititrakul (2011) [15], Kelman *et al* (2011) [17], Pu *et al* (2011) [31], Thiep *et al* (2014) [37] reported the resistance of 4.65%, 8.0%, 30.0% and 15.2%, respectively. According to Waters *et al*. (2011) [39], isolates of *Staphylococcus aureus* were found to be resistant to ampicillin, erythromycin, and penicillin-G. Tetracycline showed 56.9%, 79.0%, 69.0%, 67.0% and 61.4% resistance reported by Karpiskova *et al* (2009) [16], Jaisue and Angkititrakul (2011) [15], Kelman *et al* (2011) [17], Pu *et al* (2011) [31] and Fan *et al* (2015) [12], respectively. 100% susceptibility of ciprofloxacin against *Staphylococcus aureus* reported by Molla *et al* (2011) [24]. Kelman *et al* (2011) [17] reported 1.5% and 0.5% antibiotic resistance against gentamicin and chloramphenicol; respectively while Pu *et al* (2011) [31] reported 3% and 2% resistance only.

Conclusion

It was concluded that *Staphylococcus aureus* isolates revealed maximum sensitivity to ciprofloxacin and the sulphatriad and significant resistance to Penicillin-G and vancomycin.

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