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ORIGINAL ARTICLE

A STUDY OF COAGULAS POSITIVE STAPHYLOCOCCUS AUREUS CONTAMINATION IN CHICKEN MEAT AND INTESTINE AND INVESTIGATION OF THEIR ANTIBIOGRAM PATTERN IN EAST AZARBAYJAN OF IRAN

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ABSTRACT: The Positive Staphylococcus Aureus Coagulas is an opportunist pathogen that is able to create infection in human and animal under favorable conditions. This bacterium can grow in foodstuff and create entrotoxin that causes food poisoning in human. Since Positive Coagulas Staphylococcus Aureus is one of the common producers of food poisoning, thus, this research aims to study the degree of contamination of Positive Staphylococcus Aureus Coagulas in chicken meat and intestine and determine its antibiotic resistance. 45 samples of chicken meat and 45 samples of chicken intestine were purchased randomly from Tabriz Azar Morgh and regional markets then taken to Food stuff Health Laboratory at Islamic Azad University, Tabriz Branch. Then, the samples were made ready in accordance with the Standards 356 and 1194 of Iran Industrial Research Institute, and the Positive Staphylococcus Aureus Coagulas bacteria was recognized. The isolated bacterium was tested for antibiogram through Disk Diffusion Agar method in Muller Hinton Agar medium by means of 12 important antibiotics. Based on the biochemical test and culture of the chicken meat under examination 45 cases of 90 samples that related to chickens' meat in 27 cases (60%) Staphylococcus Aureus detected which 21 cases (77.77%) were Positive Coagulas Staphylococcus Aureus. And 45 cases of 90 samples related to chickens' intestine that in 21 cases (77.77%) Staphylococcus Aureus detected which 6 cases (28.57%) were Positive Coagulas Staphylococcus Aureus

KEYWORDS: Poultry, meat, Intestine, Staphylococcus Aureus, Antibiogram.

INTRODUCTION

The Positive Staphylococcus Aureus Coagulas is an opportunist pathogen that is able to create infection in human and animal under favorable conditions. This bacterium can grow in foodstuff and create entrotoxin that causes food poisoning in human. Sine Positive Coagulas Staphylococcus Aureus is one of the common producers of food poisoning, thus, this research aims to study the degree of contamination of Positive Staphylococcus Aureus Coagulas in chicken meat and determine its antibiotic resistance (Bergdoll, 1989; Gonzalez-Fandos *et al.*, 1994).

Staphylococcus Aureus is a facultative anaerobic, Gram-positive coccus, and is the most common cause of staph infections. It is frequently part of the skin flora found in the nose and on skin. About 20% of the human population is long-term carriers of *S. Aureus*. The carotenoid pigment staphyloxanthin is responsible for its characteristic golden colour, which may be seen in colonies of the organism. This pigment acts as a virulence factor with an antioxidant action that helps the microbe evade death by reactive oxygen species used by the host immune system (Isigidi *et al.*, 1992). Staphylococci which lack the pigment are more easily killed by host defenses (Ogston, 1984). *S. Aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia, and sepsis. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections, often causing postsurgical wound infections (Tranter, 1990; Wieneke *et al.*, 1993).

S. aureus was discovered in Aberdeen, Scotland in 1880 by the surgeon Sir Alexander Ogston in pus from surgical abscesses. Each year, some 500,000 patients in American hospitals contract a staphylococcal infection. The aim of present study was to assessment of coagulas positive Staphylococcus aureus contamination in chicken meat and intestine and investigation of their antibiogram pattern in east azarbayjan of Iran.

MATERIALS AND METHODS

45 samples of chicken meat and 45 samples of chicken intestine were purchased randomly

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from Tabriz Azar Morgh and regional markets then taken to Foodstuff Health Laboratory at Islamic Azad University, Tabriz Branch. Then, the samples were made ready in accordance with the Standards 356 and 1194 of Iran Industrial Research Institute, and the Positive Coagulas Staphylococcus Aureus bacteria was recognized. The isolated bacterium was tested for antibiogram through Disk Diffusion Agar method in Muller Hinton Agar culture medium by means of 12 important antibiotics such as Ciproflocxacin, oxacillin, Gentamycin, Tetracyclin, Erythreomycin, Trimethoprim sulfarethoxazol, Rifampcin, Vancomycin, Penicillin, Cephalothin, Claxacillin, Methicillin and lablatori test such as maltose, lecithin, Oxidase, Catalase, Coagulase, Mannitol Salt Agar and VP for detection of Coagulas Positive Staphylococcus Aureus. Also you can see some biochemical tests.

2.1. Methyl Red / Voges-Proskauer (MR/VP)

This test is used to determine which fermentation pathway is used to utilize glucose. In the mixed acid fermentation pathway, glucose is fermented and produces several organic acids (lactic, acetic, succinic, and formic acids). The stable production of enough acid to overcome the phosphate buffer will result in a pH of below 4.4. If the pH indicator (methyl red) is added to an aliquot of the culture broth and the pH is below 4.4, a red color will appear (first picture, tube on the left). If the MR turns yellow, the pH is above 6.0 and the mixed acid fermentation pathway has not been utilized (Figure 1, Right). The 2,3 butanediol fermentation pathway will ferment glucose and produce a 2,3 butanediol end product instead of organic acids. In order to test this pathway, an aliquot of the MR/VP culture is removed and a-naphthol and KOH are added. They are shaken together vigorously and set aside for about one hour until the results can be read. The Voges-Proskauer test detects the presence of acetoin, a precursor of 2,3 butanediol. If the culture is positive for acetoin, it will turn “brownish-red to pink” (Figure 1, Left). If the culture is negative for acetoin, it will turn “brownish-green to yellow” (Figure 1, Left). Note: A culture will usually only be positive for one pathway: either MR+ or VP+. Escherichia coli is MR+ and VP-. In contrast, Enterobacter aerogenes and Klebsiella pneumoniae are MR- and VP+. Pseudomonas aeruginosa is a glucose nonfermenter and is thus MR- and VP-.

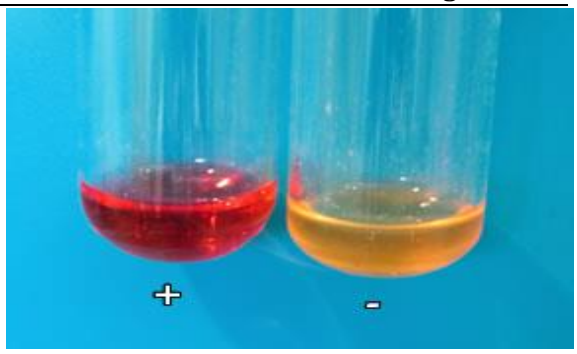

Figure 1: Methyl Red / Voges-Proskauer (MR/VP)

2.2. Coagulase test

Coagulase is an enzyme that clots blood plasma. This test is performed on Gram-positive, catalase positive species to identify the coagulase positive Staphylococcus aureus. Coagulase is a virulence factor of S. aureus. The formation of clot around an infection caused by these bacteria likely protects it from phagocytosis. This test differentiates Staphylococcus aureus from other coagulase negative Staphylococcus species (Figure 2).

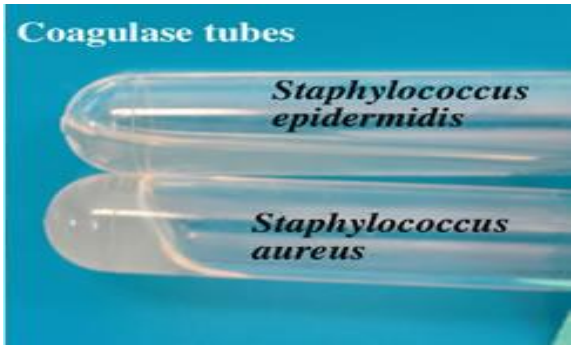

Figure 2: Coagulase test

2.3. Oxidase Test

This test is used to identify microorganisms containing the enzyme cytochrome oxidase (important in the electron transport chain). It is commonly used to distinguish between oxidase negative Enterobacteriaceae and oxidase positive Pseudomadaceae (Figure 3).

Cytochrome oxidase transfers electrons from the electron transport chain to oxygen (the final electron acceptor) and reduces it to water. In the oxidase test, artificial electron donors and acceptors are provided. When the electron donor is oxidized by cytochrome oxidase it turns a dark purple. This is considered a positive result. In the picture below the organism on the right (Pseudomonas aeruginosa) is oxidase positive.

Figure 3: Oxidase Test

2.4. Catalase Test

This test is used to identify organisms that produce the enzyme, catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O+O_2$$

The bubbles resulting from production of oxygen gas clearly indicate a catalase positive result. The sample on the right below is catalase positive. The Staphylococcus spp. and the Micrococcus spp. are catalase positive. The Streptococcus and Enterococcus spp. are catalase negative (Figure 4).

Figure 4: Catalase Test

2.5. Mannitol Salt Agar (MSA)

This type of medium is both selective and differential. The MSA will select for organisms such as Staphylococcus species which can live in areas of high salt concentration (plate on the left in the picture below). This is in contrast to Streptococcus species, whose growth is selected against by this high salt agar (plate on the right in the picture below).

The differential ingredient in MSA is the sugar mannitol. Organisms capable of using mannitol as a food source will produce acidic byproducts of fermentation that will lower the pH of the media. The acidity of the media will cause the pH indicator, phenol red, to turn yellow. Staphylococcus aureus is capable of fermenting mannitol (left side of left plate) while Staphylococcus epidermidis is not (right side of left plate). You can see some sample of Muller Hinton Agar culture medium in Figure (5) and also biochemical taste in Figure (6).

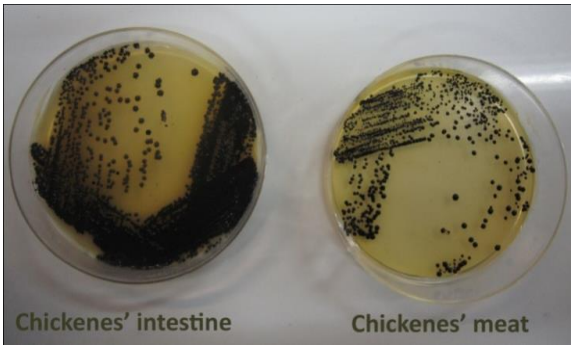

Figure 5: Muller Hinton Agar culture medium

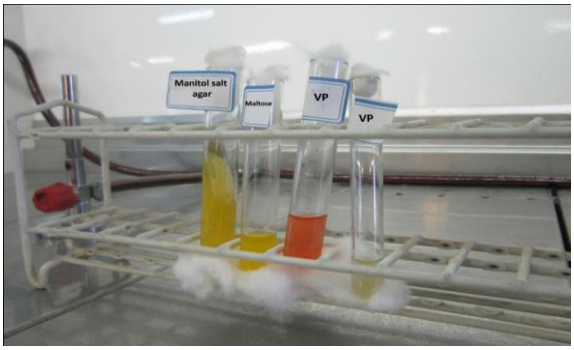

Figure 6: biochemical tests, you can see VP+ and VP-

RESULTS

Based on the biochemical test and culture of the chicken meat under examination, in chicken meat Staphylococcus Aureus was observed in 27 samples (60%) out of which 21 samples (77.77%) were related to Positive Staphylococcus Aureus Coagulas, 88.9% of the isolated were simultaneously resistant to two antibiotics (Penicillin and Methicillin), 77.8% were simultaneously resistant to one antibiotic (Colocsicillin), 66.7% were simultaneously resistant to one antibiotic (tetracillin), 44.4% were simultaneously resistant to one antibiotic (Ecsacillin) and 11.1% were resistant to five antibiotics at the same time and for the intestine in 21 samples(46.66%) it was diagnose that 6 sampels (13.33%) were contaminated with positive Staphylococcus auerus. 100% of isolate samples are resistant against three antibiotics and 85% against 1 antibiotic(Penicillin) and 42.58% against 2 antibiotics((Methicillin and Colocsicillin) simultaneously.

Table 1: result of biochemical testes on chicken meat

vp	maltose	Mannitol Salt	Coagulase	Catalase	Oxidase	lecithin	Meat
				+	+	+	1
				-	+	+	2
+	+	+	+	+	-	+	3
-	-	+	+	+	-	-	4
				-	+	-	5
				+	+	+	6
+	+	+	+	+	-	+	7
-	+	+	+	+	-	+	8
+	+	+	+	+	-	+	9
+	+	+	-	+	-	+	10
				-	+	-	11
+	+	+	+	+	-	+	12
+	+	+	+	+	-	-	13
-	+	+	+	+	-	+	14
				-	+	+	15
				-	+	+	16
+	+	+	+	+	-	-	17
				-	+	-	18
+	-	+	+	+	-	+	19
-	+	+	+	+	-	+	20
+	+	+	+	+	-	-	21
+	+	+	+	+	-	+	22
				-	+	+	23
				+	+	+	24
				-	+	+	25
+	+	+	+	+	-	-	26
				-	+	+	27
-	+	+	+	+	-	+	28
+	+	+	+	+	-	-	29
+	+	+	+	+	-	+	30
-	+	+	+	+	-	+	31
				+	+	-	32
				-	+	+	33
+	+	+	-	+	-	+	34
				+	+	+	35
-	+	+	+	+	-	+	36
-	+	+	+	+	-	+	37
				+	+	-	38
+	-	+	+	+	-	+	39
+	+	+	-	+	-	+	40
				-	+	+	41
-	+	+	+	+	-	-	42
+	+	+	+	+	-	+	43
+	+	+	+	+	-	+	44
				+	+	+	45

Table 2: result of biochemical testes on chicken intestine

vp	maltose	Mannitol Salt	Coagulase	Catalase	Oxidase	lecithin	Intestine
+	+	+	+	+	-	+	1
				-	+	+	2
				+	+	+	3
				+	+	-	4
-	+	+	+	+	-	-	5
				+	+	+	6
				+	+	+	7
-	+	+	+	+	-	+	8
				+	+	+	9
+	+	+	-	+	-	+	10
				-	+	-	11
-	+	+	+	+	-	-	12
-	+	+	+	+	-	+	13
				+	+	+	14
				-	+	+	15
				-	+	+	16
-	+	+	+	+	-	-	17
				-	+	-	18
				-	+	+	19
-	+	+	+	+	-	+	20
				-	+	-	21
+	+	+	+	+	-	+	22
+	-	+	+	+	-	+	23

				+	+	+	24
				-	+	+	25
-	+	+	+	+	-	-	26
				-	+	+	27
-	+	+	+	+	-	+	28
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-	+	+	+	+	-	+	30
-	+	+	+	+	-	+	31
-	+	+	+	+	-	-	32
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				+	+	+	35
				-	+	+	36
				+	+	+	37
				+	+	-	38
				-	+	+	39
-	+	+	-	+	-	+	40
+	-	+	+	+	-	+	41
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-	+	+	+	+	-	+	43
				+	+	+	44
				+	+	+	45

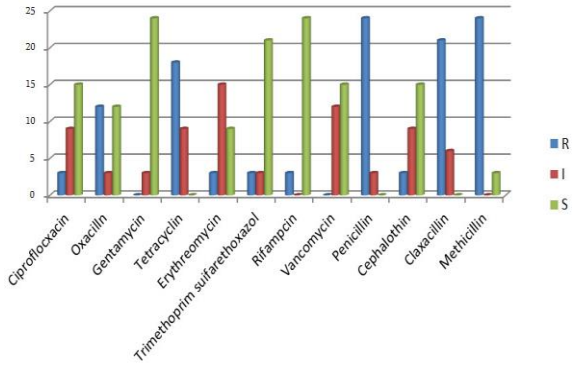

Diagram 1: result of antibiotic test on chicken meat

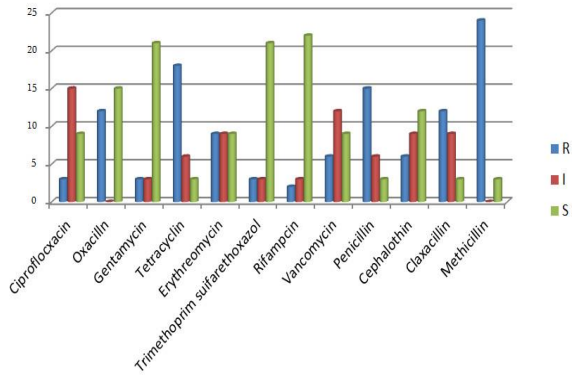

Diagram 2: result of antibiotic test on chicken intestine

DISCUSSION AND CONCLUSION

Staphylococcus aureus is considered to be one of the leading causes of food-borne illnesses. Milk, dairy products and meats are often contaminated with enterotoxigenic strains of this bacterium. Foodstuff contamination may occur directly from infected food-producing animals or may result from poor hygiene during production processes, or the retail and storage of foods, since humans may carry the microorganism (Tranter, 1990). The number of S. aureus strains that exhibits antimicrobial-resistance properties has increased, together with the potential risk of transmitting the same properties to the human microflora via foods or inducing infections hard to be treated (Normanno *et al.*, 2007). In one study that carried out by Normanno *et al.*, (2005) revealed that Meat and meat products also showed a high prevalence of contamination (10%). This contamination rate is lower than that observed in the survey previously conducted in Italy on several kinds of foodstuffs that revealed a total prevalence of coagulase positive staphylococci of 17.3%, with contamination rates ranging from 17.1% to 48.1% in meat products. In other study that accomplished by Atanassova *et al.*, (2001) demonstrated that in 25.9% of all samples S. aureus was detected by culture whereas 51.1% of the samples showed a positive result when PCR was used for the detection of the pathogen. Fresh meat was contaminated most often. By PCR, 62.2% were identified as being S. aureus positive compared to 57.7% positive samples using the cultural technique. In this research result show that, in chicken meat Staphylococcus Aureus was observed in 27 samples (60%) out of which 21 samples (77.77%) were related to Positive Staphylococcus Aureus Coagulas, 88.9% of the isolated were simultaneously resistant to two antibiotics (Penicillin and Methicillin), 77.8% were simultaneously resistant to one antibiotic (Colocsicillin), 66.7% were simultaneously resistant to one antibiotic (tetracillin), 44.4% were simultaneously resistant to one antibiotic (Ecsacillin) and 11.1% were resistant to five antibiotics at the same time and for the intestine in 21 samples(46.66%) it was diagnose that 6 sampels (13.33%) were contaminated with positive Staphylococcus auerus. 100% of isolate

samples are resistant against three antibiotics and 85% against 1 antibiotic (Penicillin) and 42.58% against 2 antibiotics (Methicillin and Colocsicillin) simultaneously.

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