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PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* ISOLATES FROM POULTRY

By

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ABSTRACT

Only fifty samples of poultry source from different farms were used in this study to isolate *Staphylococcus* species and to determine the presence of coagulase (*coa*) gene,a virulence gene detected in *S. aureus* by PCR. Primary isolation on mannitol salt agar, haemolysis, baird parker, tube coagulase test (TCT) and other biochemical characterization revealed that 24% (12/50) of the total samples were infected with staphylococci from nasal swab source and 20% (10/50) from poultry meat. Only6 isolates werebiotyped as *staphylococcus aureus* and represented as 12% from staphylococci samples from nasal swab and 4 isolates from poultry meat source represented as 8% were subjected to analysis by PCR for detection of *coa* gene. The difference in coagulase activity and recovery of *coa* gene from tested isolates indicated that there are other factor in *S.aureus*can control the coagulation activity such as vonwillbrand factor binding protein (vWbp).

INTRODUCTION

The genus *Staphylococcus* is present in skin and nasal flora and causes opportunistic infections in humans and various animals. Over the last several decades poultry is a major fast growing source of meat in the world today **Kearney (2010)**. The production and consumption of poultry meat is gradually increasing and the consumers expect safe and hygienic products without contamination with pathogenic microorganisms **Mor-Mur and Yuste (2010).** Poultry and poultry products are important vehicle for transmission of food borne pathogen **Tauxe and Blake (1992)**. *Staphylococcus* aureus is Gram-positive, facultative anaerobes, non-sporulating bacteria; most of them are recognized on the skin, mucous membranes of humans and animals and as environmental contaminants **(Feizi *et al*., 2012).** *Staphylococcus aureus* is highly vulnerable to destruction by heat treatment and nearly

all sanitizing agents. The individuals involved in the production of meat **hatakka (2006)** often attribute the presence of S. aureus in meat to inadequate hygiene during handling. Poultry meat handled in the cutting section must be stored at temperature below 7 ºC and if this temperature is exceeded, the meat must be discarded to avoid possible public health problems. **(Brazil 1998)**. In the majority of foodborne infections, it is not possible to identify the food vehicle. Poultry meat is considered as the most commonly reported foodborne pathogens vehiclefollowed by the red meat. **Hughes (2007)**. *S. aureus*is is responsible for causing a variety of animal diseases such as mastitis, arthritis and urinary tract infections and a prominent cause of food poisoning due to poor hygienic practices. *S. aureus*related food poisoning is the third largest cause of food related illness Worldwide **Sasidharan (2012)**. In almost all developing countries poor hygienic standards in poultry slaughterhouses coupled with old processing facilities, handling, transportation with significant contamination rates of market chicken products **Boonmar *et al*., (1998)**. Heavy bacterial loads enter the processing operations with the living birds and these bacteria can be disseminated throughout the plant during processing. Although normal cooking destroys *S. aureus*, recontamination can that occur during post cooking handling at the factory **Corner *et al*., (2001)**.Enterotoxin production is the lethal weapon of this pathogen. Its food poisonings commonly associated with fresh and ready-to-eat foods particularly meat products. **Aydin (2011)**. In addition to *S. aureus*, the other CoPS species can cause severe infections compared with those caused by coagulase-negative staphylococci. *Staphylococcus aureus* is one of the leading microorganisms associated with food poisoning causing outbreaks with an incidence of 11.5% **Altabari and Al-Dughaym, (2002)**.The development of PCR based method provides a promising option for the rapid identification of the pathogen and it can be used as a rapid diagnosis method. The identification of bacterial species by conventional culture method requires the days but PCR has high sensitivity and specificity and can improve the level of detection within few hours **Khan *et al*., (1998); Tamarapu *et al*., (2001)**

MATERIAL AND METHODS

Sample:

Fifty different samples, 50 poultry source were collected from different areas during November 2013 to May 2014.

Samples used were 50 nasal swabs from live birdsand poultry meat (50) from apparently healthy freshly dead carcass.

Isolation and Identification of *Staphylococcus* Species:

Pre-incubated samples (0.1 mL) in BPW were spread on the surface of Baird-Parker agar medium (Himedia, India) and supplemented with Egg Yolk Tellurite Emulsion (Himedia) and Mannitol salt agar (MSA) (Himedia). As s selective media for *S. aureus*and it was incubated further at 37 °C for 24 - 48 h. Black colonies surrounded by opaque halo on Baird-Parker agar and yellow colonies on MSA were considered presumptive *S. aureus*. Thia was confirmed with the help of Gram's staining and other biochemical tests. Gram's positive, catalase and coagulase positive *S. aureus*isolates were confirmed finally by vogus proskur test and stored frozen at-20°C innutrient broth with 15% glycerol (Difco,USA.) until molecular tests were carried.

DNA Extraction:

The bacterial genomic DNA was extracted from *S. aureus* isolates, using DNA extraction kits (Biofermentus).

Oligonucleotide primers:

Primers were selected on the basis of the 966-bp *Nuc* gene derived from the *S. aureus* Foggi strain **(Shortle, 1983)**. A set of primers were selected and applied the PCR for amplification of a sequence of the *Nuc* gene by using the two primers that targeted the gene. The sequences of the two synthetic oligonucleotide primers of 21 and 24 bases were: 5'-GCGATTGATGGTGATACGGTT-3' (forward primer) and 5;-AGCCAAGCCTTGACGAACTA AAGC-3' (reverse primer), respectively **(Brakstad *et al.,* 1992)**. *Coa* gene Primer (COAG2: CGA GAC CAA GAT TCA ACA AG; COAG3- AAA GAA AAC CAC TCA CAT CA).

PCR assay:

The reaction mixture consisted of 10 µL PCR master mix (2X) (Cat. No. K0171), 1 µL forward primer, 1 µL reverse primer, 3 µL of bacterial lysate and 5 µL of nuclease free water to make a final volume of 20 µL. The analysis was performed according to **Brakstad *et al.* (1992)** with minor modifications. A total of 40 PCR cycles were run under the following conditions: Primary denaturation at 94°C for 4 min, denaturation at 94°C for 1 min, primer annealing at 55°C for 0.5 min, extension at 72°C for 1.5 min and final extension at 72°C for 3.5 min. The PCR product was separated by electrophoresis in 1.5% agarose gel at

120V for 30 min, visualized by 500 µg mL^{-1} ethidium bromides staining, illuminated by UV transilluminator (Biometra GmBH, Germany). A 100 bp sharp DNA ladder was used as a size reference. A positive control with known *S. aureus* DNA template and a negative control (water instead of extracted DNA) were used as known standards.

RESULT

In the current study, out of total fifty poultry samples. Poultry samples were examined for the presence of pathogenic *S. aureus*. As shown in (Table 1), pure *Staphylococcus* isolates could be recovered from 12 out of 50 of total nasal samples (24%). A total of 10 from 50 samples were investigated bacteriologically for detection the occurrence of *staphylococci*. The isolation recovery rate was 20% in poultry meat samples. Results revealed that about 12% of poultry samples from nasal source and 8% from poultry meat gave typical colonies on Baird Parker Agar medium; staphylococci were isolated and identified by traditional phenotypic methods. On MSA agar, yellow colonies showing mannitol fermentation, gram staining, and catalase test were selected and coagulase test give positive result.

Table (1): Recovery rate of *staphylococcus* species from poultrysample

Source of isolates	Total sample	Total staphylococci	Recovery rate%
Nasal swabs positive for Staphylococci	**50**	**12**	**24**
Poultry meat positive for Staphylococci	**50**	**10**	**20**

Table (2):

Total staphylococci		*S.aureus* phenotypic character					
		MSA	Baired barker	Dnase	Catalase	coagulase	Voges proskaur
Nasal	**12**	**12**	**6**	**6**	**12**	**6**	**6**
Meat	**10**	**10**	**4**	**4**	**10**	**4**	**4**

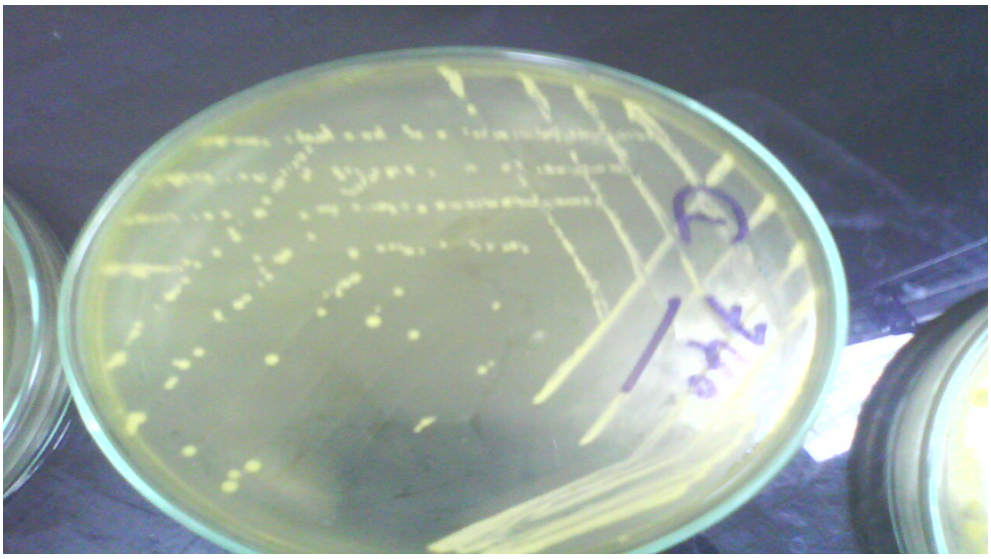

Fig (1): growth of staphylococcus on mannitol salt agar.

Fig. (2): positive coagulase test

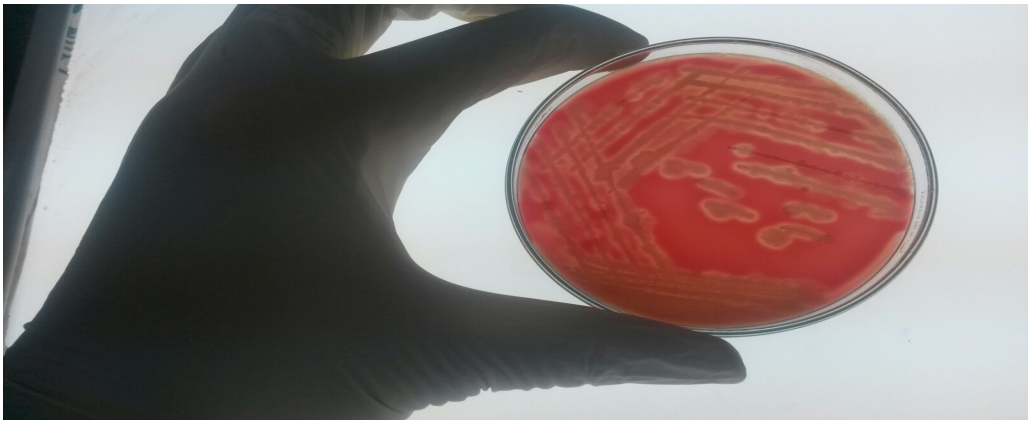

Fig. (3): showing staphylococci growth on blood agar media

Results of PCR:

From the PCR analysis 12% (6 out of 50) coagulase positive *Staphylococci* samples from nasal source and 8% (4 out of 50) from poultry meat were confirmed as *S. aureus* with *Nuc* gene 100%. The *S. aureus* strain was identified on the basis of the 270 bp PCR product corresponding to the sequence of *Nuc* gene on 1.5% agarose gel. The positive band found at the same position of positive control and no band was found in the negative control lane. PCR analysis with *coa* gene 83% from coagulase positive staphylococci nasal samples give positive result and 75% from coagulase positive staphylococci of poultry meat *coa* gene positive.

Table (3):

Staphylococcus *aureus*		***coa* gene**	**Recovery rate%**
Nasal source	**6**	**5**	**83**
Meat source	**4**	**3**	**75**

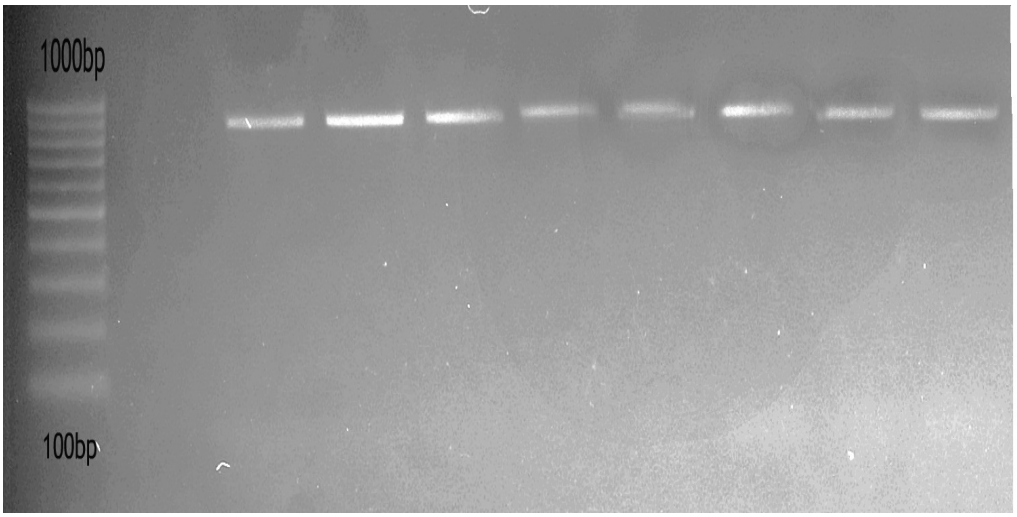

Fig. (4): Eletropheric profile of coa gene PCR products of 970 bp, for the tested staphylococcus aureus isolates, Marker 100bp DNA ladder (jena Bioscience).

DISCUSSION

This is well established facts that contaminated food is the main source of transmission for pathogenic bacteria. It is the major cause of enteric diseases in developing countries and is a major cause of mortality and morbidity. Poultry meats as a main source of foodborne in

fections have great impact in food safety. Conventional culture method generally used for the identification of *S. aureus*. In this study gram staining, catalase and coagulase tests were performed for the identification of *S. aureus* from chicken rinse samples. **Thaker *et al.* (2013)and Al-Mussawi (2014)** also performed above tests for the identification of *S. aureus*. During this study, all 50 chicken samples were cultured on mannitol Salt Agar plate (MSA) of which 12 samples were detected as positive on MSA plate from nasal swab and 10 samples from meat. Several biochemical tests are performed for confirmation of bacterial isolates in any selective media. Gram positive, cocci and arranged in irregular, grapelike clusters which are characteristics of staphylococcal species are the result of Gram staining **Holt *et al*., (1994).** All the colonies were also catalase positive. All gram and catalase positive colonies were subjected to coagulase test. Out of 12 samples 6 samples (50%)were positive in tube coagulase test and 6 samples (50%) were coagulase negative from nasal swab. and 4 coagulase positive samples from 10 (40%) and 6 coagulase negative samples(60%) from poultry meat. **Citak and Duman (2011)** found 92 (47.2%) Coagulase Positive Samples (CPS) out of 195 samples and 103 (52.8%) were coagulase negative. Generally coagulase test is considered as one kind of confirmatory test for identification of *S. aureus*. But coagulase positive samples may be the *S. intermedius* and *S. hyicus* **(Devriese *et al*., 2009). Muftah (2011)** reported 3-5% non-aureus CPS. Among 487 CPS, 82.1% were *S. aureus*, 17.7% were *S. hyicus* and 0.2% was *S. intermedius*. The prevalence rate may vary from different investigational report of different countries. **Jakee *et al.* (2008)** observed the prevalence of *S. aureus*from 223 of 370 meat samples (60.3%). **Hanson *et al.* (2011)** isolated *S. aureus* from 27 of 165 samples giving an overall prevalence of 17.8%. **Shareef *et al.* (2012)** investigated the prevalence of *Staphylococcus aureus* among US meat and poultry samples (136 samples) and found the contamination in 47%. **Kozacinski *et al.* (2006)** observed the prevalence rate of *S. aureus* was 30.30% (66 samples). Molecular (i.e., PCR based) diagnostic methods allow a better differentiation among species, serotypes. Such techniques are rapid, sensitive and specific which makes them very useful tools to improve the diagnosis and to understand the mechanisms implicated in pathogenicity, resistance and survival of the raw strains **(Tamarapu *et al*., 2001).** According to PCR results, 8 out of 120 (6.6%) coagulase positive samples were detected as *S. aureus*. **Zhang *et al*. (2012)** analyzed a total 15 strains of coagulase positive *S. aureus*. The *Nuc* primer set amplified an expected PCR product,

amplicon of 270 bp in all 15 coagulase positive isolates of all *S. aureus*. From total 120 samples 7 (5.83%) were positive in PCR. **Musa *et al.* (2009)** found 16 samples positive in PCR while 18 samples were positive in biochemical test. In this experiment, the result of different data which were found from conventional and molecular methods revealed a significant difference. The 24 out of 100 samples which were positive by biochemical method as *staphylococci,* 10 out of 100 samples recoverd as *staphylococcus aureus* with phenotypic character and *nuc* genebut only 8 samples were detected as *S. aureus* by *coa* gene PCR. **Musa *et al.* (2009)** found similarity in biochemical tests and PCR method. Two*staphylococcus aureus* isolates give negative result with *coa* gene **McAdow *et al.* (2012)** stated that two staphylococcal products, the canonical coagulase (coa) as well as the recently identified vonwillbrand factor binding protein (vWbp), promote similar modifications of the coagulation cascade during host infection.

CONCLUSION

This study is the document for the identification of *S. aureus* in chicken collected from different farmers though bacteriological methods are used for the detection of pathogen but PCR is more accurate and specific for the confirmation of pathogen. From the comparison of above mentioned result it has been revealed that the prevalence of *Staphylococcus aureus* infections in farmers is alarming for future because of its epidemiological importance. Proper management should be taken in the farmer and supermarkets to minimize the zoonotic disease transmissible from chicken meat to humans.

REFERENCES

Altabari, G. and A.M. Al-Dughaym, (2002): The role of sanitary inspection of meat in relation of food poisoning. Proceedings of the 2nd Annual Scientific Meeting for Environment Hygiene, (Meat Hygiene), (ASMEH'02), Riyadh, Saudi Arabia, pp: 180-203.

Aydin, A.; Sudagidan, M. and Muratoglu K. (2011): Prevalence of Staphylococcal enterotoxins, toxin genes and genetic-relatedness of foodborne *S. aureus* strains isolated in the Marmara Region of Turkey. Int JFood Microbiol; 148: 99-106.

Boonmar, S.; Bangtrakulnonth, A.; Pornrunangwong, S.; Marnrim, N.; Kaneko, K. and Ogawa, M. (1998): i>Salmonella in broiler chickens in Thailand with special reference to contamination of retail meat with Salmonella enteritidis. J. Vet. Med. Sci., 60: 1233-1236.

Brakstad, O.G.; Aasbakk, k. and Maeland, J.A. (1992): Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the*nuc* gene. J. Clin. Microbiol, 30: 1654-1660.

Brazil (1998): Ministerio da Agricultura, Pecuaria e samples in Turkey: Prevalence and antimicrobial resistance. Abastecimento, Portarian 210, de 10 de novembro de 1998. Journal of Food, Agricultur and Environment, 9 (1): 156 -158.

Carbas B.; Cardoso L. and Coelho AC. (2010): Investigation on the knowledge associated with foodborne diseases in consumers of north eastern Portugal. Food Control 2012; 30 (1): 54-5.

Citak, S. and Duman, T. (2011): *Staphylococcus aureus* and coagulase- negative Staphylococcus from raw chicken samples in Turkey: Prevalence and antimicrobial resistance. J. Food Agri. Environ., 9: 156-158.

Corner, D.E.; Davis, M.A. and Zang, L. (2001): Poultry-Borne Pathogens II: Plant Considerations. In: Poultry Meat Processing, Sams, A.R. (Ed.). Chapter 9, CRC Press LCC, Boca Raton, FL., USA, and ISBN-13: 9781420042177, pp: 137-158.

Devriese, L.A.; Hermans, K.; Baele M. and Haesebrouck, F. (2009): *Staphylococcus pseudintermedius* versus *Staphylococcus intermedius*. Vet. Microbiol, 133: 206-207.

Feizi, A.; Nazeri, M. and Pilevar, A. (2012): Isolation of *Staphylococcus* spp. genera from broiler breeder flocks in East Azerbaijan Province of Iran: Prevalence and antimicrobial susceptibility. Afr.J.Microbiol.Res, 6:5819-5823.

Hatakka, M., Björkroth, K.J., Asplud, K., Maki-Petays, N. 25. Kozacinski, L., Hadziosmanovic, M. and Zdolec, N. (2006): Genotypes and enterotoxicity of Microbiological quality of poultry meat on the Croatean Staphylococcus aureus isolated from the hands and nasal market. VeterinarskiArchiv, 76 (4): 305-313.

Hanson, B.M., A.E. Dressler, A.L. Harper, R.P. Scheibel and S.E. Wardyn *et al*., (2011): Prevalenceof *Staphylococcusaureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. J. Infect. Public Health, 4: 169 -174.

Holt, J.G., N.R. Kreig, P.H.A. Sneath, J.T. Staley and S.T. Williams, (1994): Bergey's Manual of Determinative Bacteriology. 9th Edn, Lippincott Williams and Wilkins, Baltimore, USA, ISBN-13: 9780683006032, Pages: 787.

Hughes C, Gillespie IA, O'Brien SJ: The breakdowns in food safetygroup. Foodborne transmission of infectious intestinal disease inEngland and Wales, 1992-2003. Food Control 2007; 18: 766-772.

Jakee, J., A.S. Nagwa, M. Bakry, S.A. Zouelfakar, E. Elgabry and W.A. Gad El-Said, (2008): Characteristics of *Staphylococcus aureus* strains isolated from human and animal sources. Am-Euras. J. Agric. Environ. Sci., 4: 221-229.

Kearney, J., (2010): Food consumption trends and drivers. Philos. Trans. R. Soc. B: Biol. Sci., 365: 2793-2807.

Khan, M.A.; Kim, C.H.; Kakoma, I.; Morin, E. and Hansen, R.D. (1998): Detection of Staphylococcus aureus in milk by use of polymerase chain reaction analysis. Anim. J. Vet. Res., 59: 807-813.

Kozacinski, L., M. Hadziosmanovic and N. Zdolec, (2006): Microbiological quality of poultry meat on the Croatian market. Vet. Arhiv, 76: 305-313.

Lowy, F.D. (1998): Staphylococcus aureus infections. N. Engl. J. Med., 339: 520-532.

McAdow, M.; Missiakas, D.M. and Schneewind, O. (2012): Staphylococcus aureus secretes coagulase and von Willebrand factor binding protein to modify the coagulation cascade and establish host infections. J Innate Immun, 4 (2): p. 141-8.

Mor-Mur, M. and J. Yuste, (2010): Emerging bacterial pathogens in meat and poultry: An overview. Food Bioprocess Technol., 3: 24-35.

Muftah, H.H.B., (2011): Identification and antimicrobial resistance of *Staphylococcus aureus* from bovine milk. Master Thesis, Ohio State University, Veterinary Preventive Medicine.

Musa, N.O.; Eltom, K. Gessler, F.; Bohnel, H.; Babiker, A.; Elhaj, M.O. and S.M. El Sanousi, (2009): Characterization of some *Staphylococcus aureus* subspecies anaerobius isolates by spa and coa genes. J. Anim. Vet. Adv., 8: 2272-2275.

Salehi, T.Z. and Bonab, S.F. (2006): Antibiotics susceptibility pattern of *Escheichia coli* strains isolated from chickens with colisepticemia in Tabriz Province, Iran. Int. J. Poult. Sci., 5: 677-684.

Sasidharan, S.; Prema, B. and Yoga Latha L. (2011): Antimicrobial drug resistance of *S. aureus* in dairy products. Asian Pac J TropBiomed: 1(2): 130-132.

Shortle, D., (1983): A genetic system for analysis of staphylococcal nuclease. Gene, 22: 181-189.

Shareef, A.M.; Farag, R.A. and Al-Ruthwani, E.K. (2012): Evaluation of bacterial load of frozen chicken thighs in Mosul markets. Iraq J. Vet. Sci., 26: 63-69.

Songer, J.G. and Post, K.W. (2005): Veterinary microbiology. High contamination of *S.aureus* is the principal reason E1slst eEvidenr. InBca, c.and fungal agents of animal disease. For the inadequate microbiological quality of chicken.

Tamarapu, S.; McKillip, J.L. and Drake, M. (2001): Development of a multiplex polymerase chain reaction assay for detection and differentiation of *Staphylococcus aureus* in dairy products. J. Food Prot., 64:664-668.

Tauxe, R.V. and Blake, P.A. (1992): Epidemic cholera in Latin America. J. Am. Med. Assoc., 267: 1388-1390.

Thaker, H.C.; Brahmbhatt, M.N. and Nayak, J.B. (2013): Isolation and identification of *Staphylococcus aureus* from milk and milk products and their drug resistance patterns in Anand, Gujarat. Vet. World, 6: 10-13.

Zhang, T.; Wang, C.; Wei, X.; Zhao, X. and Zhong, X. (2012): Loop-mediated isothermal amplification for detection of *Staphylococcus aureus* in dairy cow suffering from mastitis. J. Biomed. Biotech. 10.1155/2012/435982.