LWT - Food Science and Technology 90 (2018) 152–156

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

# Pathogenicity and antibiotic resistance of coagulase-negative staphylococci isolated from retailing chicken meat

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ARTICLE INFO

*Keywords:*
Coagulase-negative staphylococci
Raw chicken meat
Pathogenicity
Antibiotic resistance

ABSTRACT

Diverse coagulase-negative staphylococci (CNS) species isolated from meat are previously reported and considered as potential pathogenic staphylococci through obtain horizontal transferred elements (e.g. toxic, antibiotic resistance factors) from other bacteria. In this study, prevalence, homology and pathogenicity of CNS isolated from 180 raw chicken meat samples in retail markets were studied. The overall prevalence of CNS was 11.7% and centralized in 69.2% of *Staphylococcus epidermidis* and 30.8% of *Staphylococcus warneri* among diverse sources (including different retail settings, suppliers and products). Molecular typing showed highly homogenous patterns in the same species. Antibiotic susceptibility test revealed a common penicillin and erythromycin resistance among all of CNS, while 17 *S. epidermidis* isolates displayed multidrug resistance of gentamycin (GM)-kanamycin (K)-penicillin (P)-erythromycin (E), except one methicillin-resistant *S. epidermidis* isolate which was typed in staphylococcal cassette chromosome *mec* (SCC*mec*) V. Conclusively, diverse sourced raw chicken meat products exhibited a centralized prevalence of *S. epidermidis* and *S. warneri*, and a highly homogenous of genome in the same CNS specie. Though the negative toxigenicity of CNS was determined, constant multidrug resistance in *S. epidermidis* and P-E resistance in CNS need to be highly concerned regarding human health.

## 1. Introduction

CNS in foods are earlier regarded as food-associated saprophytes (Becker, Heilmann, & Peters, 2014; Fijalkowski, Peitler, & Karakulska, 2016), which are normally inhabited on skin and mucous membrane to multiply as non-infectious bacterium (Piette & Verschraegen, 2009) or contributed to the fermentation of sausages (Blaiotta et al., 2004; Sánchez Mainar, Matheuse, De Vuyst, & Leroy, 2017), particularly, as previously studied (Becker et al., 2014; Ruiz, Barragan, Sesena, & Palop, 2016), *S. epidermidis* is commonly reported as predominant CNS among diverse food-related settings. Many authors have suggested that CNS can possess or acquire mobile pathogenic factors (e.g. exotoxins, enterotoxins, adherence factors, leukocidins and antibiotic resistance) in the forms of transposons, pathogenicity islands (PIs), plasmids and phages, similar to *Staphylococcus aureus*, turn into opportunistic food-borne pathogen causing severe infection or food poisoning, to date, correlative genotypic and phenotypic characterizations were determined as previously reported (Arciola, Baldassarri, & Montanaro, 2001; Bhargava & Zhang, 2014; Cunha, Peresi, Calsolari, & Araújo Júnior, 2006; Fijalkowski et al., 2016). Currently, the methods of determination of CNS are developed from classic biochemical isolation to molecular identification (e.g. specific DNA sequencing and protein characterizing) (Fijalkowski et al., 2016; Huber et al., 2011). Furthermore, as previously described, PFGE (golden molecular typing standard) and random amplified Polymorphic DNA-polymerase chain reaction (RAPD-PCR) are commonly used for molecular typing of CNS (Bhargava & Zhang, 2014; Iacumin, Comi, Cantoni, & Cocolin, 2006; Kawano et al., 1996).

Antibiotic resistance of CNS is an emerging important concern regarding public health in the past decades, constant works have confirmed the prevalent antibiotic resistance of CNS isolated from animal-origin foods. (Bhargava & Zhang, 2014; Fijalkowski et al., 2016; Kawano et al., 1996; Perillo et al., 2012; Wang et al., 2013). It is demonstrated that CNS serve as a reservoir for *mecA* which encoding penicillin-binding protein 2a (PBP2a) (Bhargava & Zhang, 2014; Huber et al., 2011; Wang et al., 2013), and it is hypothesized that methicillin-resistant CNS is responsible for the emergence of methicillin-resistant *S. aureus* (MRSA) which pose a serious life threat during infections. In recent years, routine antibiotic (penicillin, erythromycin and tetracycline etc.) resistances are confirmed among many CNS species

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https://doi.org/10.1016/j.lwt.2017.12.029
Received 20 July 2017; Received in revised form 6 December 2017; Accepted 11 December 2017
Available online 12 December 2017
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(Yurdakul, Erginkaya, & Ünal, 2013), especially for penicillin resistance which indicated a common existence of *bla* gene that encode β-lactam (Huber et al., 2011). Moreover, multidrug resistance is becoming more frequent due to CNS obtain multi mobile elements of antibiotic resistance.

Technically, raw chicken meat products are processed through regular feeding, slaughtering and transporting, whereas, the abuse usage of antibiotics and inadequate handling might cause unexpected antibiotic resistance and cross-contamination of CNS. Therefore, it is important to investigate the prevalence and pathogenicity of CNS in retailing raw chicken meat products, so as to provide the current background information of CNS in raw chicken meat. In this study, identification, molecular typing, toxic gene existence, antibiotic resistance and biofilm-forming ability of CNS were determined.

## 2. Materials and methods

### 2.1. Identification of CNS

A total of 180 raw chicken meat products (60 cuts (including drumstick, wing middle joints and entire wings), 60 breasts, 60 whole carcasses) were randomly selected from different brands (including well known and unknown brands) and supermarkets, samples were placed in sterilized bags and transported to the laboratory on ice immediately. Sample enrichment were prepared as previously described (Bhargava & Zhang, 2014; Fijalkowski et al., 2016) with slight modification as follow, a 20 g surface portion of meat was aseptically weighted and homogenized in 180 ml 0.1% (w/v) peptone water with 10% (w/v) NaCl and incubated for 12 h at 37 °C, followed by selection of 3–5 suspected colonies (black colony without transparent circle surrounded) on Baired-Parker Agar supplement with 5% egg-yolk and tellurite (both bought from Beijing Landbridge Biotech, China). Rabbit coagulase and blood test were performed to confirm the coagulase and hemolytic status. VITEK2 automated system (BioMerieux, France) was used for species identification. DNA was extracted using commercial bacteria DNA extraction kit (Tiangen Biotech, China). Finally, 16S rRNA and 23S rRNA amplicons were sequenced for species confirmation (Perillo et al., 2012).

### 2.2. Molecular typing methods

Original PFGE experiment for CNS isolates showed that this typing method is indistinguishable among the same species, additionally, *S. warneri* isolates showed an extremely resistance to lysostaphin enzymolysis in gel plug, therefore, we employed RAPD-PCR for the further typing as several studies reported (Ruiz, Izquierdo, Sesena, & Palop, 2008; Ruiz et al., 2016), briefly, performing 35 cycles of 94 °C for 1 min, 37 °C for 1 min and 72 °C for 2 min. Bionumeric (Applied-Math Co., Ltd, USA) software was used for dendrogram analysis by means of unweighted pair group method with arithmetic average (UPGMA). Reproducibility of RAPD-PCR was evaluated by three independent experiments.

### 2.3. Staphylococcal pathogenic genes characterization

The staphylococcal toxic and partial antibiotic resistant genes were detected by PCR reaction, including enterotoxin gene series: *a, b, c, d, e g, h, i, j, k, l, m, n, o, p, q, r, u, tsst-1* (encoding toxic shock syndrome toxin), *pvl* (encoding panton-valentine leukocidin), *eta, etb* (encoding exfoliatin toxins), *icaA*, *icaD* (responsible for biofilm-forming). Unique antibiotic resistant genes (*bla, mecA*) detection was carried out to validate the antibiotic resistance testing. Methicillin-resistant CNS isolate was typed according to composition of SCC*mec* assembles as previously described (D. C. Oliveira & Lencastre, 2002). Primers were listed in Table 1.

**Table 1**
Primers used in this study.

| Gene | Sequence (5′–3′) | Reference |
|---|---|---|
| *16S* | CCACCTTCCTCCGGTTTGTCACC<br>AACTCTGTTATTAGGGAAGAA | (Perillo et al., 2012) |
| *coa* | ACCACAAGGTACTGAATCAACG<br>TGCTTTCGATTGTTCGATGC | (Perillo et al., 2012) |
| *nuc* | TGAAGTCAAATAAATCGCTTGC<br>CCCTTTTCCACTAATTCCTTATTGT | (Perillo et al., 2012) |
| *sea* | CCTTTGGAAACGGTTAAAACG<br>TCTGAACCTTCCCATCAAAAAC | (Omoe et al., 2002) |
| *seb* | TGTATGGTGGTGTAACTGAGCA<br>CCCGTTTCATAAGGTGAGTTGT | This study |
| *sec* | CTCAAGAACTAGACATAAAAGCTAGG<br>TCAAAATCGGATTAACATTATCC | (Perillo et al., 2012) |
| *sed* | GTGGTGAAATAGATAGGACTGC<br>ATATGAAGGTGCTCTGTGG | (Perillo et al., 2012) |
| *see* | CTGGAGGCACACCAAATAAA<br>TCCGTGTAAATAATGCCTTGC | This study |
| *seg* | AAGTAGACATTTTTGGCGTTCC<br>AGAACCATCAAACTCGTATAGC | (Omoe et al., 2002) |
| *seh* | CAACTGCTGATTTAGCTCAG<br>GTCGAATGAGTAATCTCTAGG | (Perillo et al., 2012) |
| *sei* | CAACTCGAATTTTCAACAGGTACC<br>CAGGCAGTCCATCTCCTG | (Perillo et al., 2012) |
| *sej* | TGCACCTCCTCTCTGCGCCT<br>AGTGCATTGTAACGCCCCCGT | This study |
| *sek* | TAGGTGTCTCTAATAATGCCA<br>TAGATATTCGTTAGTAGCTG | (Perillo et al., 2012) |
| *sel* | GCTTTCTGGAAGACCGTATCCTGTG<br>GGCGATGTAGGTCCAGGAAACCT | (Perillo et al., 2012) |
| *sem* | ATGCTGTAGATGTATATGGTCTAAG<br>CGTCCTTATAAGATATTTCTACATC | (Li et al., 2015) |
| *sen* | ATGAGATTGTTCTACATAGCTGCAAT<br>AACTCTGCTCCCACTGAAC | (Li et al., 2015) |
| *seo* | TGTAGTGTAAACAATGCATATGCAAATG<br>TTATGTAAATAAATAAACATCAATATGATGTC | (Li et al., 2015) |
| *sep* | TTAGACAAACCTATTATCATAATGG<br>TATTATCATGTAACGTTACACCGCC | (Li et al., 2015) |
| *seq* | AAGAGGTAACTGCTCAAG<br>TTATTCAGTCTTCTCATATG | (Li et al., 2015) |
| *ser* | AAACCAGATCCAAGGCCTGGAG<br>TCACATTTGTAGTCAGGTGAACTT | (Li et al., 2015) |
| *seu* | TAAAATAAATGGCTCTAAAATTGATGG<br>ATCCGCTGAAAAATAGCATTGAT | (Li et al., 2015) |
| *tsst-1* | GCTTGCGACAACTGCTACAG<br>TGGATCCGTCATTCATTGTTAT | (Perillo et al., 2012) |
| *pvl* | ATCATTAGGTAAAATGTCTGGACATGATCCA<br>GCATCAAGTGTATTGGATAGCAAAAGC | (Li et al., 2015) |
| *mecA* | TGGCTATCGTGTCACAATCG<br>CTGGAACTTGTTGAGCAGAG | (Vazquez-Sanchez, Lopez-Cabo, Saa-Ibusquiza, & Rodriguez-Herrera, 2012) |
| *bla* | ACTTCAACACCTGCTGCTTTC<br>TGACCACTTTTATCAGCAACC | (Li et al., 2015) |
| *icaA* | TCTCTTGCAGGAGCAATCAA<br>TCAGGCACTAACATCCAGCA | (Arciola et al., 2001) |
| *icaD* | ATGGTCAAGCCCAGACAGAG<br>CGTGTTTTCAACATTTAATGCAA | (Arciola et al., 2001) |
| *eta* | CTAGTGCATTTGTTATTCAA<br>TGCATTGACACCATAGTACT | (Johnson et al., 1991) |
| *etb* | ACGGCTATATACATTCAATT<br>TCCATCGATAATATACCTAA | (Johnson et al., 1991) |

### 2.4. Biofilm formation in vitro

Biofilm formation in *vitro* was evaluated as previously described (Peeters, Nelis, & Coenye, 2008; Ruiz et al., 2016) with slight modification. Briefly, after 24 h cultivation at 37 °C in 96-well polystyrene microtiter plate, each well washed with PBS (pH 7.4) for three times, the adherent biofilm immobilized with methanol for 15 min and wells air-dried at room temperature, 0.5% (w/v) crystal violet staining processed for 5 min, then tap water used to wash the redundant dye, each well air-dried to spare water, the attached dye resolved with 33% (v/v)

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