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Staphylococcal Poisoning Foodborne Outbreak: Epidemiological Investigation and Strain Genotyping

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1 **Topic of the paper.** Tracing the source of a staphylococcal food poisoning

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4 **Full title of the paper.** Staphylococcal poisoning foodborne outbreak: epidemiological investigation and strain
5 genotyping

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34 **Abstract**

35 In June 2011, an outbreak of *Staphylococcus aureus* enterotoxin food poisoning gastroenteritis occurred in Turin,
36 Italy, following a catered dinner party given at a private home. Within a few hours, 26 of the 47 guests
37 experienced gastrointestinal illness and 9 were hospitalized. A retrospective cohort study using a standardized
38 questionnaire was carried out and the risk ratios (RR) for each food item were calculated. The analysis indicated
39 consumption of seafood salad as the most probable cause of the outbreak (RR = 11.72, 95% confidence interval
40 [CI], 1.75-78.54). Biological samples were collected from 4 of the hospitalized guests (stool and vomit), nasal
41 mucosa swabs from 3 food handlers employed with the caterer and available food residuals. All stool and vomit
42 samples tested positive for enterotoxigenic *S. aureus*. As residues of the seafood salad were no longer available
43 for sampling, suspected contamination could not be verified. However no other food was found contaminated by
44 *S. aureus* or its enterotoxins. All isolates from the biological samples were characterized at genomic level by
45 means of two multiplex PCR protocols to determine the presence of genes encoding staphylococcal enterotoxins
46 (SEs), pulsed-field gel electrophoresis (PFGE) and staphylococcal protein A gene (*spa*) typing to describe their
47 genetic profiles. All the isolates presented genes encoding SEA and SEI; the PFGE genetic profiles revealed the
48 same pulsotype in the microorganism isolated from the hospitalized guests as in one of the isolate from the nasal
49 mucosa of one of the food handlers and their *spa* typing analysis reported two closely related *spa* types (t701 and
50 t267), implicating the food handler as the most likely outbreak source.

51

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53 Staphylococcal food poisoning (SFP) is one of the most common foodborne diseases worldwide (23). According
54 to the European Food Safety Authority (EFSA), outbreaks of foodborne illnesses in the European Union in 2010
55 totalled 5262, of which 461 were caused by bacterial toxins produced by *Staphylococcus aureus*, *Bacillus*, or
56 *Clostridium*, the fourth most common causative agent in foodborne outbreaks in Europe. In particular, 274 of
57 these foodborne outbreaks were caused by staphylococcal enterotoxins (SEs) (6).

58 SFP is a foodborne intoxication that develops after the ingestion of food contaminated with enterotoxigenic *S.*
59 *aureus* strains, generally improperly prepared or stored (16). *S. aureus* is a coagulase-positive staphylococcus
60 (CPS), a facultative Gram-positive anaerobe which can grow over wide range of pH (4-10, optimally at a pH of
61 6-7), temperatures (7-48°C), and water activity (minimum, 8.83). Some strains are able to produce specific
62 thermoresistant enterotoxins with a somewhat narrower production range (11). *S. aureus* colonizes both humans
63 and domestic animals. As common opportunistic pathogen, it colonizes the skin and mucosa of humans and
64 animals, with nasal carriage rates between 30 and 50% in the adult population (9). Skin infections,
65 nasopharyngeal or oropharyngeal staphylococcal or streptococcal secretions also have been frequently linked to
66 worker-associated outbreaks (18;19).

67 But *S. aureus* is not only a commensal colonizer. It can cause serious infections, toxinoses and life-threatening
68 diseases, including skin and soft tissue infections, toxic shock syndrome and septicemia (20). The growth of *S.*
69 *aureus* in foods makes it a potential public health hazard, and many strains are able to produce heat-stable SEs
70 that cause food poisoning if ingested (2;3;4). Symptoms of foodborne illness include copious vomiting, diarrhea,
71 and abdominal pain or nausea (13;15). The severity of the illness depends on the amount of toxin present in the
72 ingested food and the general health of the victim (17). While foodborne illness symptoms subside
73 spontaneously after 24 h in most cases, fatality rates range from 0.03% in the general population to 4.4 % in
74 children and the elderly (5).

75 In the present study we describe a local SE foodborne outbreak in which we used statistical and microbiological
76 methods to identify the likely cause. To do this, we investigated for the source of the outbreak by comparing the
77 isolates obtained from the residual food and those from the biological samples of symptomatic patients and food
78 handlers.

79

80

Material and methods

81 On the evening of 19 June 2011, 9 persons presented at the emergency room of two hospitals in the province of
82 Turin because of abdominal pain and gastrointestinal symptoms (vomit and diarrhea) . All reported having
83 attended a catered dinner party, together with 38 other guests, at a private home. An epidemiological
84 investigation was carried out to determine the full extent of the outbreak and its probable source. Biological and
85 food residual samples were collected and microbiological analyses performed.

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Epidemiological investigation

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All 47 dinner guests were contacted and administered a structured questionnaire by the Local Health Authority to collect information on food exposure and illness symptoms. The guests self-reported what food exposures they had and whether or not they became ill following the exposure. Information about sex, age, onset and nature of symptoms, and duration of illness were collected. From these data, the main symptoms and the time of onset were analyzed to determine the possible causes of the outbreak and to draw the epidemic curve. A retrospective cohort study was then conducted: the questionnaire responses were entered into a database, analyzed with Epi

93 Info™ 3.5, and the specific attack rate was calculated for each type of food served at the dinner party.
94 To measure the association between the food served and the illness symptoms, adjusted Mantel-Haenszel
95 estimates of the risk ratio (RR) with a 95% confidence interval (CI) for each food item were calculated. A P
96 value less than 0.05 was considered statistically significant.

97 *Laboratory investigation*

98 Stool and emesis samples were collected from 4 of the 9 hospitalized dinner guests and analyzed for pathogenic
99 bacteria and toxins potentially responsible for the reported symptoms. Analyses for *C. perfringens* enterotoxin, *S.*
100 *aureus*, and *B. cereus* were performed. Stool samples were also tested for *Salmonella* spp., *Shigella* spp.,
101 *Campylobacter* spp., Verotoxigenic *E. coli* (VTEC), *Vibrio* spp., *Yersinia enterocolitica* and *Listeria*
102 *monocytogenes*. In addition, nasal swabs were collected from the 3 food handlers employed with the caterer and
103 involved in the preparation of the food served at the dinner. *S. aureus* strains isolated from emesis, stool and
104 nasal swab samples were sent to the Italian National Reference Laboratory for CPS (NRL for CPS - Turin) for
105 the detection of genes encoding SEs by multiplex PCR (Method of the EU-RL CPS, Version 1, October 2009).
106 The strains were sent to the European Reference Laboratory (EURL for CPS - ANSES France) for molecular
107 characterization by PFGE. The pulsotypes were compared using BioNumerics® Software (Applied Maths).
108 Finally these isolates were analyzed with *spa* typing method (10). *S. aureus* protein A polymorphic region
109 was amplified using forward and reverse primers, *spa-1113f* (5'-AAAGACGATCCTTCGGTGAGC-3')
110 and *spa-1514r* (5'-CAGCAGTAGTGCCGTTTGCTT-3'). Sequences analysis was performed
111 using BioNumerics® Software (Applied Maths), whereas numeric *spa* repeats and *spa* type codes
112 were assigned through the Ridom StaphType server (Ridom GmbH, Germany).

113 Food samples were collected and sent to the Istituto Zooprofilattico of Piemonte, Liguria and Valle d'Aosta (IZS)
114 for enumeration of CPS (ISO 6888-2), *B. cereus* (ISO 7932), and *C. perfringens* (ISO 7937). The samples were
115 also analyzed for *Salmonella* spp. (ISO 6579), SEs (EU-RL Method, Ver. 5, 2010), and *B. cereus* diarrheal toxin
116 (Internal Method).

117

118

118 **Results**

119 *Epidemiological and clinical characteristics of cases*

120 All 47 dinner guests completed the questionnaire (response rate, 100%) and 26 met the case clinical criteria
121 definition, yielding an overall attack rate of 55%. The 26 cases reported the following clinical symptoms:
122 vomiting (85%), diarrhea (77%), abdominal pain (69%), nausea (62%), epigastralgia (31%), chills (27%),
123 headache (8%), sweating (7%) and bitterness in the throat (7%). Nine of these 26 (34%) guests presented at the
124 hospital emergency room because of the severity of symptoms.

125 First case clinical symptoms started 1 hour and 30 minutes after the dinner and the last case occurred 10 hours
126 and 30 minutes after the meal (Fig. 1). The median incubation period was 4.25 hours; the duration of illness
127 ranged from 30 minutes to 36 hours (median, 8 hours and 10 minutes).

128 *Food risk assessment*

129 The data obtained through the questionnaires on food items consumed at the dinner all pointed to the seafood
130 salad as the most likely source of the outbreak: the attack rate for persons who ate the meal was 78% and the RR
131 was 11.72 (95% CI, 1.75-78.54; Mantel-Haenszel: 20.65 $p \leq 0.0005$).

132

132 *Laboratory investigation*

133 Stool and emesis samples from 4 of the 9 hospitalized dinner guests were analysed. All the biological samples
134 tested negative for *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *C. perfringens*, *B. cereus*, VTEC, *Vibrio*
135 spp., *Yersinia enterocolitica* and *Listeria monocytogenes*. Faeces and emesis samples tested positive for
136 enterotoxigenic *S. aureus*. A total of 14 *S. aureus* were isolated from stool and emesis samples of the 4 patients
137 (Table 1). All 14 isolates tested with the two multiplex PCR protocols showed the same enterotoxin gene profile,
138 presenting genes encoding for SE types A and I (Fig. 2 a and b).

139 The nasal swab samples collected from the 3 food handlers involved in the dinner preparation tested positive for
140 *S. aureus* (Fig. 3 a and b) carrying the enterotoxin gene profile shown in Table 2.

141 The PCR results confirmed that the *S. aureus* isolated from the nasal mucosa of one food handler (no. 3) carried
142 the same encoding sequences (*sea* and *sei*) as those deriving from the 14 biological samples of the patients.

143 In order to characterize at the genomic level of the 15 isolates and to determine their genetic correlation, we
144 carried out PFGE and *spa* typing analysis. With PFGE method the 15 isolates were found to belong to the same
145 clade, with a 100% degree of homology between their profiles (Fig. 4). The presence of the same genetic band
146 pattern, as analyzed with BioNumerics® software, confirmed the genetic correlation between the *S. aureus*
147 isolates. According to *spa*-typing, the 15 isolates were grouped into the same comparisons database and two *spa*
148 types was assigned: t701 for 14 isolates (93.33% degree of homology) including 13 patients biological samples
149 and *S. aureus* isolated from the nasal mucosa of one food handler (no. 3). The other isolate deriving from
150 biological sample resulted in t267 *spa* type.

151 Microbiological analyses of the food residues other than the seafood salad (no more of which was available)
152 showed a CPS concentration below the limit of quantification (LOQ) of the method; no significant positivity for
153 other microbiological contaminations was found in the food samples.

154

155

Discussion

156

157 The results of the present investigation shed light on the epidemiological and genetic aspects of this foodborne
158 illness outbreak. One of the criteria for confirmation of a staphylococcal food poisoning outbreak is isolation of
159 an organism belonging to the same strain from stool or vomit from two or more ill people (21). Microbiological
160 analysis permitted to isolate enterotoxigenic *S. aureus* from the stool and emesis samples of the hospitalized
161 cases and from the nasal swabs of the food handlers employed with the caterer. The *S. aureus* isolates were
162 evaluated using PCR methods to identify the SE genes. Multiplex PCR allows to identify numerous SE targets
163 when other more traditional methods, such as ELISA, are unavailable for identifying all toxins. PCR
164 demonstrated that the 15 isolates, 14 from the patients' biological samples and one from the nasal mucosa of 1
165 food handler, carried the same SE encoding genes (*sea* and *sei*). The presence of the *sea* gene in *S. aureus*
166 deriving from food matrices, and its encoded toxin, is in line with previous data (7;8).

167 This study confirms that the combined use of molecular methods would be a practical tool to match clinical
168 isolates with food isolates and/or swabs. Furthermore, such an approach would help to identify potential
169 outbreaks that would otherwise not be possible using traditional microbiological methods. *S.aureus* isolates
170 (n=15) resulted to belong at t701 and t267 *spa*-types: they own similar repeats (10 units), differing in number of
171 one repeating unit (figure 5) Patients biological samples isolates (n=13) and *S. aureus* isolated from the nasal
172 mucosa of one food handler (no. 3) belong to the same *spa* type (t701), already reported in a case of outbreak

173 (22), whereas the other one belong to t267 *spa* type. PFGE characterization, currently considered the gold-
174 standard technique for molecular typing of isolates deriving from a suspected common source (1), allowed us to
175 determine the genetic relatedness of the 15 *S. aureus* isolates (100% homology degree), providing convincing
176 evidence about the source of food contamination. Because of the *spa* type percentage of similarity is based on
177 the analysis of repeated units of shared or analogous sequences, 88% of similarity degree permits to consider
178 genetically related two or more strains. Matching molecular data permits to confirm epidemiological correlation
179 between patients strains and food handler. Due to the different discriminatory power of the two techniques, as
180 demonstrated in a previous study (22), there is the possibility to have the same PFGE patterns but different *spa*
181 types.

182 In contrast, microbiological analysis of the residual food samples tested negative for *S. aureus*. However, the
183 statistic epidemiological analysis of food risk assessment demonstrated a significant attack rate for the seafood
184 salad that was no longer available for sampling for microbiological analysis.

185 The molecular characterization provided epidemiological evidence and the microbiological testing of the
186 biological samples allowed us to identify as the possible source of contamination an enterotoxigenic *S. aureus*
187 strain deriving from one of the food handlers employed with the caterer. Although the study lacks complete
188 microbiological data for all food samples, the results offer clues that the seafood salad could have been
189 contaminated with *S. aureus* strains able to synthesize SEA and SEI toxins in that food matrix. The growth of
190 enterotoxigenic *S. aureus* strains to more than 10^6 cells per g of food is generally considered necessary to produce
191 a sufficient amount of enterotoxin to cause foodborne intoxication (14). As demonstrated in a previous study,
192 (12) food temperature is one of the most important factors that affect SEs production: the study demonstrated
193 that the amount of SE is positively correlated to the growth of *S. aureus* at various storage temperatures, except
194 for 17°C.

195 Food safety authorities require that food processors identify and control steps critical to food safety: good
196 practice codes (Good Manufacturing Practices [GMP] and Good Hygienic Practices [GHP]) comprise the
197 fundamental principles, procedures, and means for ensuring an environment is suitable for safe food production
198 (24). GMP programmes cover both the safety and quality aspects of food production, including GHP and Hazard
199 Analysis and Critical Control Points (HACCP). GHP comprises the essential principles of food hygiene
200 applicable throughout the food chain so that food is safe and suitable for human consumption, while the HACCP
201 system identifies, evaluates, and controls hazards relevant for food safety. A solid GHP system is a prerequisite
202 to implementing HACCP. Our study underscores the importance of applying GHP in each step of food treatment
203 and the presence of *S. aureus* as a common opportunistic pathogen in the food chain.

204

205

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208

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272 **Figure legends**

273

274 **FIGURE 1.** Epidemic curve of the outbreak.

275

276 **FIGURE 2 (a).** Enterotoxin gene profiles of *S.aureus* strains isolated from emesis and stool of patients. Agarose gel
277 electrophoresis of multiplex PCR products for detection of *sea* to *see* and *ser* genes: lane M, marker (50-bp ladder); lanes 1-3,
278 strains from emesis of patient n.1; lanes 4-6, strains from emesis of patient n. 2; lanes 7-8, strains from emesis of patient n. 3;
279 lane 9-10-11-12, positive controls; lanes 13-15, strains from stool of patient n. 3; lanes 16-18, strains from stool of patient
280 n. 4; lane 19, negative control (free of DNA). **(b).** Enterotoxin gene profiles of *S.aureus* strains isolated from emesis and stool
281 of patients. Agarose gel electrophoresis of multiplex PCR products for detection of *seg* to *sej* and *sep* genes: lane M, marker
282 (50-bp ladder); lanes 1-3, strains from emesis of patient n. 1; lanes 4-6, strains from emesis of patient n. 2; lanes 7- 10,
283 strains from emesis of patient n. 3; lane 8-9, positive controls; lanes 11-13, strains from stool of patient n. 3; lanes 14-16,
284 strains from stool of patient n. 4; lane 17, negative control (free of DNA).

285

286 **FIGURE 3 (a).** Enterotoxin gene profiles of *S.aureus* strains isolated from nasal mucosa of food handlers. Agarose gel
287 electrophoresis of multiplex PCR products for detection of *sea* to *see* and *ser* genes: lane M, marker (50-bp ladder); lane 1,
288 strain from food handler n. 1; lane 2, strain from food handler n. 2; lane 3, strain from food handler n. 3; lane 4-5-6-7,
289 positive controls; lane 8, negative control (free of DNA). **(b).** Enterotoxin gene profiles of *S.aureus* strains isolated from
290 nasal mucosa of food handlers. Agarose gel electrophoresis of multiplex PCR products for detection of *seg* to *sej* and *sep*
291 genes: lane M, marker (50-bp ladder); lane 1, strain from food handler n. 1; lane 2, strain from food handler n. 2; lane 3,
292 strain from food handler n. 3; lane 4-5, positive controls; lane 6, negative control (free of DNA).

293

294 **FIGURE 4.** PFGE profiles of *S.aureus* isolated strains. Lane M, reference strain (*S.aureus* NCTC 8325); lanes 1-3, strains
295 from emesis of patient n.1; lanes 4-6, strains from emesis of patient n. 2; lanes 7 and 8, strains from emesis of patient n. 3;
296 lanes 9-11, strains from stool of patient n.3; lanes 12-14, strains from stool of patient n. 4; lane 15, strain from nasal mucosa
297 of food handler n. 3.

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Table 1. Number of *S. aureus* strains isolated from emesis and stools of patients.

Patient	Emesis samples (strain number)	Stool samples (strain number)
No.1	3	
No. 2	3	
No. 3	2	3
No. 4		3

302
303
304
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Table 2. Enterotoxin gene profile of *S. aureus* isolated from food handlers.

Food handlers	Multiplex PCR protocol no.1						Multiplex PCR protocol no.2				
	<i>sea</i>	<i>seb</i>	<i>sec</i>	<i>sed</i>	<i>see</i>	<i>ser</i>	<i>seg</i>	<i>seh</i>	<i>sei</i>	<i>sej</i>	<i>sep</i>
No. 1							pos		pos		pos
No. 2								pos			
No. 3	pos									pos	

307

Figure 1

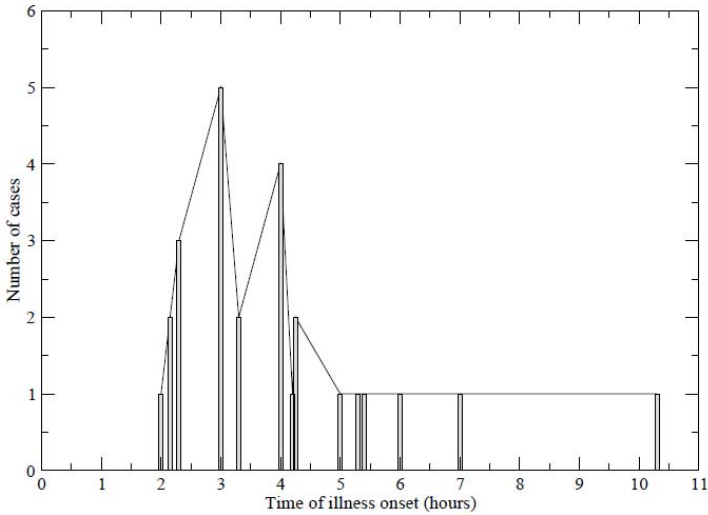


Figure 2 (a)

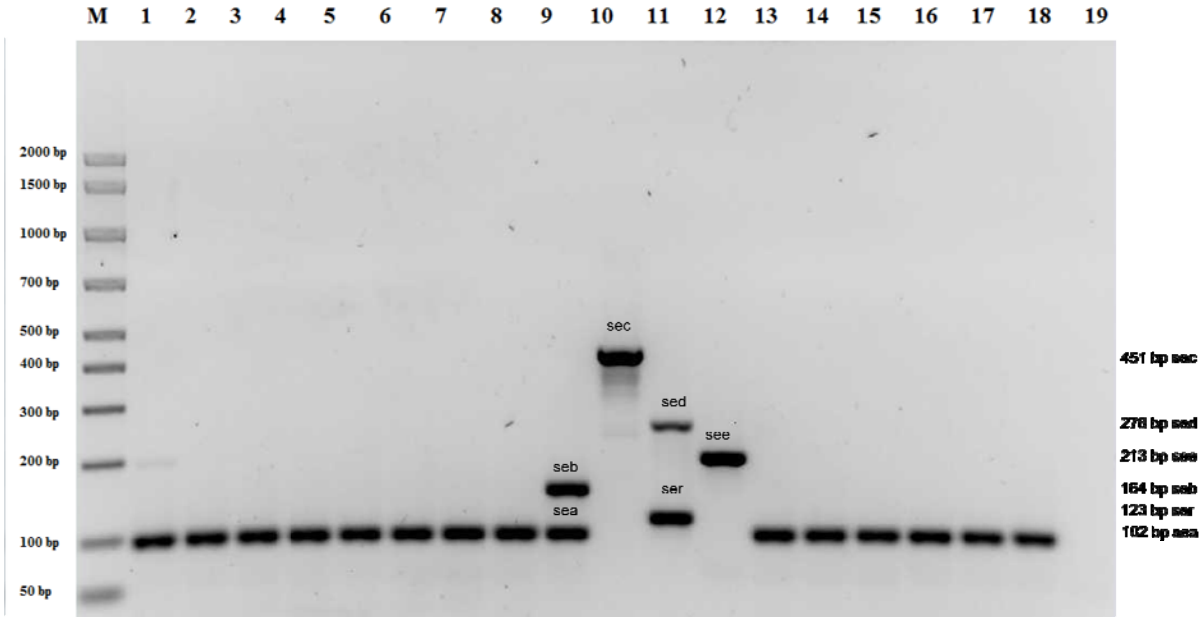


Figure 2 (b)

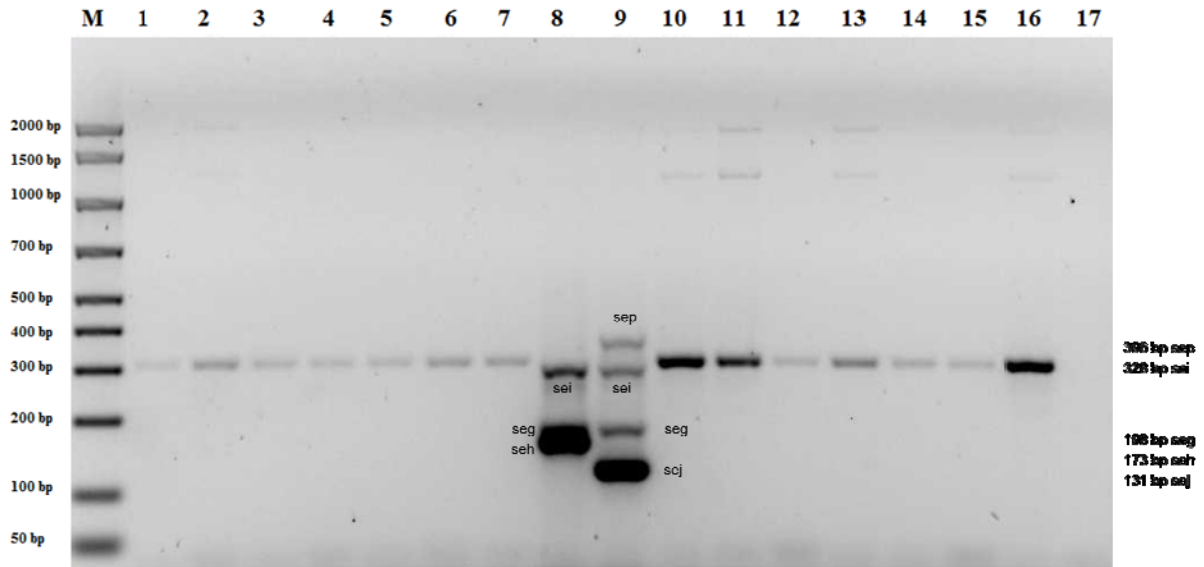


Figure 3 (a)

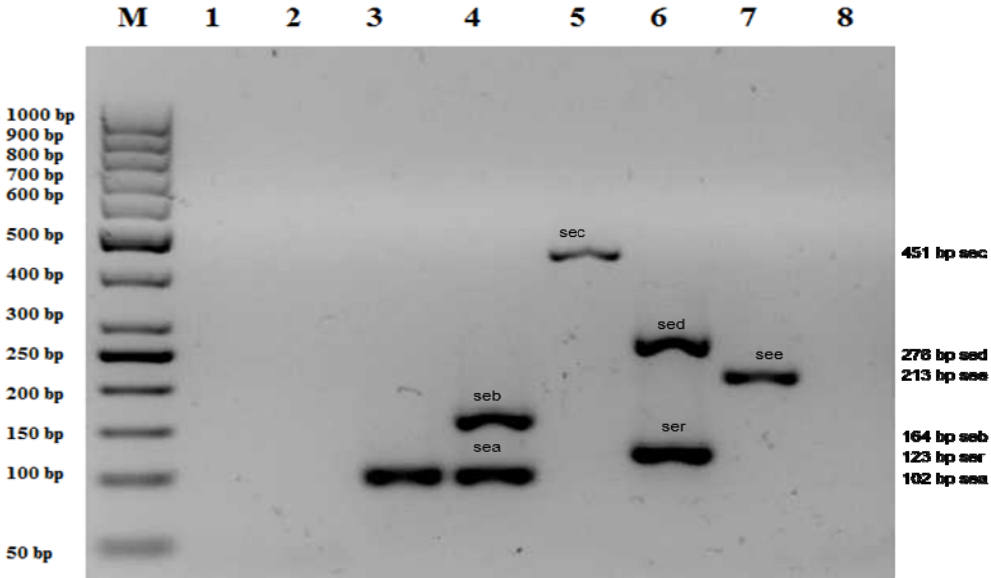


Figure 3 (b)

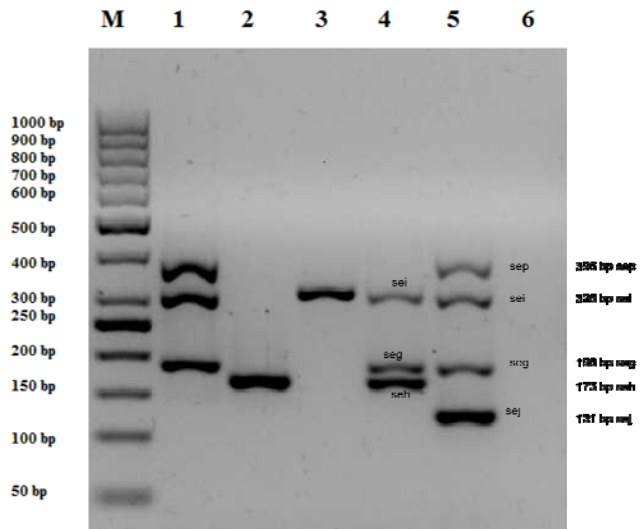


Figure 4

