Phenotypic Characterization of *Staphylococcus aureus* and *Escherichia coli* Isolated from Some Bivalve Molluscus in Egypt

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Abstract: A total of 100 samples of bivalve molluscus were subjected for enumeration, isolation and identification of *Staphylococcus aureus*, total coliforms, faecal coliforms and *Escherichia coli*. 61 strains of *S. aureus* were isolated, 20 strains from each yellow and black Gandoufly and 21 strains from Om El-Kholoul. While 36 strains of *E. coli* were isolated 13 strains from yellow Gandoufly, 12 strains from black Gandoufly and 11 strains from Om El-Kholoul. All *S. aureus* isolates were coagulase positive. The antimicrobial sensitivity test revealed that all the *S. aureus* isolates were sensitive to rifampicin, vancomycin, cephalexin, cefataxime, chloramphenicol, kanamycin, neomycin, amikacin, ciprofloxacin, enrofloxacin, ofloxacin, oxacillin, cloxacillin, amoxy-clavulenic acid and ampicillin. Meanwhile they were resistant to pefloxacin and flumequine, but only 92% of the isolates to streptomycin. Concerning *E. coli* all isolates were resistant to ampicillin, amoxicillin and streptomycin, however only 50% of them were resistant to trimethoprim-sulfamathoxazole, lincomycin, neomycin and pefloxacin. All *E. coli* isolates were sensitive to ofloxacin, enrofloxacin, gentamicin, spiramicin and amikacin. Moreover 93.3, 90, 90, 83.3, 80, 80, 76.6, 63.3 and 60% of the isolates were sensitive to cephataxime, cephalexin, amoxy-clavulenic, chloramphenicol, tetracycline, kanamycin, flumequine, ciprofloxacin and erythromycin respectively.

Key words: Bivalve · *S. aureus* · *E. coli* · Antimicrobial sensitivity

INTRODUCTION

Nowadays there is a global increase in the consumption of seafood leading to a significant global problem concerning hazards of seafood borne pathogens [1]. Seafood borne diseases associated with consumption of shellfish are the major challenge to the food hygienists in the 21st century [2]. Bivalve molluscus: oysters, mussels and clams are filter feeding which can accumulate pathogenic bacteria and toxic metals at levels higher than those in their surrounding waters [3-5].

It is worthy to mention that *Staphylococcus aureus* (*S. aureus*) was the most prevalent seafood borne pathogens detected in the seafood [6-8]. *Staphylococcus aureus* causes superficial skin infections and life-threatening diseases such as endocarditis, sepsis and soft tissue, urinary tract, respiratory tract, intestinal tract, bloodstream infections [9-12].

*E. coli* occurrence in seafood is considered a sanitary case and may represent a risk to the consumers if related to pathogenic strains, especially diarrhea genic *E. coli*. However, the presence of non-pathogenic *E. coli* in fish and shellfish should be recognized as an indicator of fecal contamination and presence of other enteric pathogens [13]. Fecal coliforms remain the standard indicator of choice for fish and shellfish harvest waters meanwhile, *E. coli* is used to indicate recent fecal contamination or unsanitary processing [14].

In humans, *Escherichia coli* can cause a variety of intestinal and extra-intestinal infections, such as diarrhea, urinary tract infection, meningitis, peritonitis, septicemia and gram-negative bacterial pneumonia [15].

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Bacteria originating from food animals frequently carry resistance to a range of antimicrobial agents, including those commonly used in humans which could be attributed to the heavy use of antimicrobial agents in food animal production; moreover these bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other bacteria of human clinical significance [15-16].

A little information is available about *S. aureus* and *E. coli* among bivalve mollusks found in Egypt, so the aim of the present study is to isolate *S. aureus* and *E. coli* from some bivalve molluscs found in Alexandria and El behaira governorate markets and also to throw light on their antibiogram.

**MATERIALS AND METHODS**

**Samples:** A total of 100 samples of bivalve molluscs [33 yellow Gandoufly, 33 black Gandoufly (Tapes decussatus) and 34 Om El-Kholoul (Wedge clam, Donax trunculus)] were collected from different local markets of Alexandria and El behaira. Representing each of 13 live and 20 chilled for Gandoufly and 13 live and 21 chilled for Om Elkholoul.

**Preparation of Molluscus Homogenate:** Ten grams from each sample were homogenized with 90 ml ¼ ringer’s solution [17]. One ml from the sample original homogenate was added to a test tube containing 9 ml ringer’s solution to provide a dilution of 10^2. Similarly a ten tenfold serial dilutions were prepared [17].

**Enumeration of Total Staphylococcal Count:** 0.1 ml from each dilution was spread over a dry surface of double sets of Baird Parker agar plate (black shining convex colonies, 1-1.5 mm in diameter with narrow white margin and surrounded by a clear area extending into opaque medium) were enumerated and the average number per gram was calculated [18].

**Isolation and Biochemical Identification of *Staphylococcus aureus***: Five suspected colonies from typical and atypical *S. aureus* colonies on Baird Parker medium were picked up, purified and then transferred to soft agar tubes for preservation and further identification [18].

**Antibiotic Sensitivity Test (S. aureus):** The disk diffusion technique was used to perform the antimicrobial susceptibility test for *S. aureus* isolates using amikacin, ampicillin, amoxicillin, oxacillin, amoxicillin, ofloxacin, enrofloxacin, pefloxacin, flumequine, (amoxy+clavulanic acid), ciprofloxacin, erythromycin, spiramicin, neomycin, kanamycin, gentamicin, chloramphenicol, streptomycin, trimethoprim-sulfamathoxazole, cephataxime, cephalaxine, bacitracin, tetracycline, rifampicin and vancomycin. Zone diameter of inhibition was measured among the antimicrobial agents used and interpretation for results was recorded [19].

**Enumeration of Total Coliforms Count:** The most probable number (MPN), 3-tubes dilutions technique was used [18]. Three tubes of Laurylesulphatetryptose (LST) broth supplemented with inverted Durham’s tubes per dilution were inoculated with 1ml of each dilution. All LST broth tubes showed gas productions within 48 hours were recorded as positive. Confirmed by subcultured into brilliant green lactose bile broth incubated at 37°C for 48 hours. Tubes showed gas after 48 hours were recorded as positive. Calculated from MPN tables for 3- tubes dilutions recommended by [18].

**Enumeration of Fecal Coliforms Bacteria:** A loopful from each positive LST broth was inoculated into LST broth supplemented with inverted Durham’s tubes. Incubated at 44.5 ± 0.5 c for 24 – 48 hours in a thermostatically control water bath. Positive tubes showing turbidity and gas production were calculated according to the recommended tables [20].

**Isolation and Biochemical Identification of *Escherichia coli***: A loopful from each gas positive LST broth was streaked on to plates of Eosine Methylene blue (EMB) agar incubated at 37°C for 24 hours. Five suspected colonies from typical (greenish metallic with sheen and black purple center) and atypical *E. coli* colonies on EMB agar medium were picked up, purified and then transferred to soft agar tubes for preservation and further identification [17].

**Antibiotic Sensitivity Test (E. coli):** The disk diffusion technique was used to perform the antimicrobial susceptibility test for *E. coli* isolates using amikacin, ampicillin, amoxicillin, ofloxacin, enrofloxacin, pefloxacin, flumequine, lincomycin, (amoxy+clavulanic acid), ciprofloxacin, erythromycin, spiramicin, neomycin, kanamycin, gentamicin, chloramphenicol, streptomycin, oxacillin, cephalexine, trimethoprim-sulfamathoxazole, cephalaxine and tetracycline, Zone diameter of inhibition was measured among the antimicrobial agents used and interpretation for results was recorded [19].
RESULTS AND DISCUSSION

Data shown in Table (1) revealed that *S. aureus* was isolated from all chilled molluscus, but not found in fresh live molluscus. This is may be due to contamination during the collection operation, which is increased by handling of the product by the salesman [21]. The minimum *S. aureus* count was $2 \times 10^2$, $2 \times 10^4$ and $3 \times 10^5$cfu/g for yellow gandoufly, black gandofly and OM-Elkholoul chilled samples respectively, however maximum count was $3.1 \times 10^5$, $1.7 \times 10^5$ and $4.6 \times 10^5$ cfu/g for the same samples respectively (Table 1). Nearly similar results were recorded by Mansour et al. [22]. Table 2 declares that isolated strains of *S. aureus*, were mannitol fermentative. Such results agree with that achieved before Mansour et al. [22] and disagree with the results recorded by Ezzeldeen et al. [23] for *S. aureus* strains from Egyptian salted fish. Moreover, 91.81% of the isolates were betahemolytic and 8.19% isolates were alpha hemolytic. Similar results were obtained by Ata [24], who found that all of the *S. aureus* isolates obtained in his study had hemolytic activities on sheep blood agar. However, such results disagree with that of Ezzeldeen et al. [23] who reported that the majority of *S. aureus* isolates were non hemolytic (62.7%) on sheep blood agar.

It is obvious from Table 2 that all *S. aureus* isolates were 100% catalase and O/F positive, which agrees with the findings of Ata [24] and Ezzeldeen et al. [23]. Concerning the coagulase activity, it was evident that 100% of *S. aureus* isolates were coagulase positive. Nearly similar results were obtained by Ata [24] but disagree with that obtained by Vilhelmsson et al. [8] who found that 25% of the isolated *S. aureus* were coagulase positive.

Results explained in Table 3 clearly indicated that all the *S. aureus* isolates showed 100% sensitivity to vancomycin, ciprofloxacin, enrofloxacin, ofloxacin, gentamicin, spiramycin, amikacin, neomycin, kanamycin, chloramphenicol, ampicillin, amoxy-clavulenic acid, cloxacillin, oxacillin, cephalaxin, cephalixin and rifampicin, 80% to penicillin G and bacitracin, 72% amoxicillin, 68% trimethoprim-sulfamathoxazole, 64% erythromycin, 60% tetracycline and 8% streptomycin, on the other hand the isolates were 100% resistant to pefloxacin and flumequine.

Concerning vancomycin, similar data were reported by Tiwari, et al. [25] and Eok et al. [26]. However, Ezzeldeen et al. [23] recorded that only 91.5% of *S. aureus* isolates were sensitive to vancomycin. Ciprofloxacin sensitivity was 100% and several other studies achieved nearly the same results [23, 27]. On the contrary, Parmar et al. [28] reported that 48.57% of *S. aureus* isolates were sensitive to ciprofloxacin. In this study 100% of *S. aureus* isolates were sensitive to enrofloxacin, this agrees with...
Dendani et al. [29]. However Parmar et al. [28] reported that 71.43% of S. aureus isolates were sensitive to enrofloxacin. Also in our study all S. aureus isolates were sensitive to ofloxacin, this result is nearly similar to Eok et al. [30].

Furthermore, our result showed that 100% of S. aureus isolates were susceptible to gentamicin, this is agrees with data of Dendani et al. [29]. All S. aureus isolates were sensitive to neomycin. Attia [31] showed that 44.9% of the S. aureus isolates were sensitive to neomycin. Also all of S. aureus isolates were sensitive to amikacin which is nearly similar to what reported by Sotirova et al. [32]. Also in our result indicated that all S. aureus isolates were sensitive to spiramicin. Dendani et al. [29] found that no resistance to spiramicin among S. aureus isolated strains.

All S. aureus isolates were sensitive to kanamycin and this is nearly similar to data of Caracappa et al. [33]. Also all S. aureus isolates were sensitive to chloramphenicol, similarly to results of Rossetti [34]. Furthermore our results showed that all S. aureus isolates were sensitive to oxacillin in agreement with Rossetti [34] and Gentilini et al. [35] who found that all S. aureus isolates were susceptible to oxacillin. All S. aureus isolates were sensitive to cloxacillin and ampicillin. Such results disagrees with Jha et al. [36] and Turutoglu et al. [37]. Moreover, S. aureus isolates were sensitive to cephalexine and this is nearly is similar to results of Singh et al. [38], while Tiwari et al. [25] found that 55.5% of S. aureus isolates were resistant to cephalexine. Also S. aureus isolates were sensitive to cephataxime which nearly agrees with Ozsan et al. [39].

Table 4 declares that the total percentage of E. coli isolates were 39% from yellow gandoufly, 36% from black gandoufly and 32% from OM-Elkholoul, this result nearly agrees with Fusco et al. [43] who examined 59 bivalve shellfish, 16 of them (27%) were positive for E. coli. Some strains of Escherichia coli are highly pathogenic and cause diseases include dysentery, pneumonia and meningitis De Vinney et al. [44]. Gastroenteritis caused by E. coli may be related to fecal contamination in the extraction and harvesting areas of bivalve molluscs, or the lack of appropriate handling practices [45, 46].

Our result clearly indicated that all E. coli isolates showed extreme resistance to ampicillin, amoxicillin and streptomycin. However all the isolates were sensitive to gentamicin, enrofloxacin, ofloxacin, spiramicin and amikacin, while 93.3% of them were sensitive to cephataxime, 90% to cephalexin and amoxy-clavulanic, 83.3% to chloramphenicol, 80% to kanamicin and tetracycline, 76.6% to flumequine, 63.3% to ciprofloxacin, 60% to erythromycin and 50% to pefloxacine, neomycin, lincomycin and trimethoprim-sulfamathoxazole (Table 6).

Popovic and Popara [47] and Roy et al. [48] found that all their E. coli isolated strains showed resistance against ampicillin. However Giurov [49] and DaCoasta et al. [50] reported that only 22.9% of the isolates were resistance to ampicillin. In addition all E. coli isolates were resistant to amoxicillin, this result agrees with that obtained by Zhang et al. [51] who showed that all isolates were extremely resistant to amoxicillin. In contrast Saha et al. [52] and Nazir et al. [53] concluded a much lower percent of resistance. Also E. coli isolates were resistant to streptomycin and such data are similar to that of Wani et al. [54] and Smith et al. [55].

Gentamicin sensitivity was 100% for all E. coli isolates, similar result was acheived by Filali et al. [56]. Our data revealed that all of E. coli strains were sensitive to enrofloxacin, Asawy and Abd El-Latif [57] conclude that all E. coli strains were sensitive to enrofloxacin. Lower results were detected by others [58-60]. On the other hand results showed that all E. coli isolates were sensitive to

<table>
<thead>
<tr>
<th>Positive E. coli isolates</th>
<th>Yellow gandoufly</th>
<th>Black gandoufly</th>
<th>OM_Elkholoul</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow gandoufly</td>
<td>13</td>
<td>0</td>
<td>39.39</td>
</tr>
<tr>
<td>Live chilled</td>
<td>20</td>
<td>13</td>
<td>65%</td>
</tr>
<tr>
<td>Black gandoufly</td>
<td>13</td>
<td>0</td>
<td>36.36</td>
</tr>
<tr>
<td>Live chilled</td>
<td>20</td>
<td>12</td>
<td>60%</td>
</tr>
<tr>
<td>OM_Elkholoul</td>
<td>13</td>
<td>0</td>
<td>32.35</td>
</tr>
<tr>
<td>Live chilled</td>
<td>21</td>
<td>11</td>
<td>52.3%</td>
</tr>
</tbody>
</table>
ofloxacin and this is in accordance with that obtained by Chah et al. [61]. Concerning amikacin E. coli isolates explored sensitivity to itsimilar to results of Li et al. [62].

Mean while, 93.3 and 90% of E. coli isolates were sensitive to cephataxime and cephalexine (cephalosporins) respectively. Such results are similar to what obtained by Zhang et al. [51] and nearly similar to what achieved by Saha et al. [52] showed that sensitivity of E. coli isolates was to cephataxime (79.17%), but Abou-Dobara et al. [63] stated that only 26% of the E. coli isolates were sensitive to cephataxime.Moreover 90% of E. coli isolates were sensitive to amoxy-clavulunicacid, this is similar to Luis et al. [64]. Also our study revealed that 83.3% of E. coli isolates were sensitive to chloramphenicol.

In addition 80% of E. coli isolates were sensitive to kanamycin. Meanwhile Giurov [49] achieved similar result, Popovic and Popara [47] and Gundogan et al. [65] reported that 80.7% of E.coli strains were resistant to kanamycin.

Nearly 80% of E. coli isolates were sensitive to tetracycline and this result is nearly similar toDa Coasta et al. [50] who found that 27.6% of E. coli strains were resistant to tetracycline, However Roy et al. [48] and Zhang et al. [51] declares that all E. coli isolates were 100% resistant to tetracycline.Our study showed that 76.6% of E. coli isolates were sensitive to flumequine (fluroquinolones) and this result nearly agrees with Saleem et al. [59] and Giurov [49]who stated that 80% of E. coli isolates were sensitive to flumequine. On the contrary, Li et al. [62] observed that 57.1–66.7% of E. coli strains were resistant to fluroquinolones.

About 63.3% of E. coli isolates were sensitive to ciprofloxacain (fluroquinolones). Similar data recorded by Luis et al. [64] but not with that gained by Li et al. [62]. It was found that 60% of E. coli isolates were sensitive to erythromycin. Saha et al. [52] declares that 66.67% of E. coli isolates were resistant to erythromycin, but Wani et al. [54] stated that all of the E. coli isolates were resistant to erythromycin.

Trimethoprim-sulfamathoxazole (sulfonamides) susceptibility of E. coli isolates was 50%. Smith et al. [55] concluded that 50 to 100% of E. coli isolates were resistance to drugs such as sulfonamides. However, Saleem et al. [59] recorded a much smaller sensitivity of E. coli isolates to trimethoprim-sulfamathoxazole.

REFERENCES


