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# 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.* 61strains 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, cephataxime, chloramphenicol, kanamycin, neomycin, amikacine, spiramicin, gentamicin, ciprofloxacin, enrofoxacin, ofloxacin, oxacillin, cloxacillin, amoxy-clavulinic 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 andamikacin. Moreover 93.3, 90, 90, 83.3, 80, 80, 76.6, 63.3 and 60% of the isolates were sensitive to cephataxime, cephalexin, amoxy-clavulinic, 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 molluscus 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 molluscus [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 and13 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 mlfrom 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 drysurface 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, penicillin G, cloxacillin, oxacillin, amoxicillin, ofloxacin, enrofloxacin, pefloxacin, flumequine, (amoxy+clavulanic acid), ciprofloxacin, erythromycin, spiramicin, neomycin, kanamycin, gentamicin, chloramphenicol, streptomycin, trimethoprim-sulfamathoxazole, cephataxime, cephalexine, 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, enrofloxacin, pefloxacin, flumequine, lincomycin, (amoxy+clavulanic acid), ciprofloxacin, erythromycin, spiramicin, neomycin, kanamycin, gentamicin, chloramphenicol, streptomycin, ofloxacin, cephataxime, trimethoprim-sulfamathoxazole, cephalexine 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 did 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 $2\times10^4$, $2\times10^4$ and $3\times10^4$cfu/g for yellow gandoufly, black gandofly and OM-Elkholoul chilled samples respectively, however maximum count was $3.1\times10^5$, $1.7\times10^5$ and $4.6\times10^5$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%) onsheep 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 ofAta [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 3clearly indicated that all the *S.aureus* isolates showed 100% sensitivity tovancomycin, ciprofloxacin, enrofloxacin, of loxacillin, gentamicin, spiramicin, amikacin, neomycin, kanamycin, chloramphenicol, ampicillin, amoxy-clavulinic acid, cloxacillin, oxacillin, cephataxime, cephalexin 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% andseveral 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

Table 1: Prevalence and *Staphylococcus aureus* count among different molluscus samples

| | | *S. aureus* isolates | | *S.aureus cfu/g* | | |
|---|---|---|---|---|---|---|
| Samples | Positive | No | % | Mean | Min | Max |
| Yellow gandoufly | live | 0 | 0 | | $<10^2$ | $<10^2$ |
| | chilled | 20 | 100 % | $7.6\times10^4$ | $2\times10^4$ | $3.1\times10^5$ |
| Black gandoufly | live | 0 | 0 | | $< 10^2$ | $< 10^2$ |
| | Chilled | 20 | 100% | $6\times10^4$ | $2\times10^4$ | $1.7\times10^5$ |
| OM-Elkholoul | live | 0 | 0 | | $< 10^2$ | $< 10^2$ |
| | Chilled | 21 | 100% | $1.63\times10^5$ | $3\times10^4$ | $4.6\times10^5$ |

Table 2: Biochemical and some enzyme production characteristics of *S. aureus*isolates

| Biochemical test | Results | No | % |
|---|---|---|---|
| Catalase | positive | 61 | 100 |
| O/F test | positive | 61 | 100 |
| Coagulase | positive | 61 | 100 |
| Mannitol fermentation | Fermentative | 61 | 100 |
| Haemolysis on sheep blood agar | Β haemolysis | 56 | 91.8 |
| | αQ haemolysis | 5 | 8.19 |

Table 3: Antibiotic resistance of *S.aureus* isolates (n=25)

| | Sensitive | | Resistant | |
|---|---|---|---|---|
| Antibiotic | No | % | No | % |
| Ampicillin | 25 | 100% | 0 | 0% |
| Penicillin G | 20 | 80% | 5 | 20% |
| Amoxy-clavulinic acid | 25 | 100% | 0 | 0% |
| Cloxacillin | 25 | 100% | 0 | 0% |
| Oxacillin | 25 | 100% | 0 | 0% |
| Amoxicillin | 18 | 72% | 7 | 28 |
| Ofloxacin | 25 | 100% | 0 | 0% |
| Enrofloxacin | 25 | 100% | 0 | 0% |
| Ciprofloxacin | 25 | 100% | 0 | 0% |
| Pefloxacin | 0 | 0% | 25 | 100% |
| Flumequine | 0 | 0% | 25 | 100% |
| Gentamicin | 25 | 100% | 0 | 0% |
| Spiramicin | 25 | 100% | 0 | 0% |
| Amikacin | 25 | 100% | 0 | 0% |
| Streptomycin | 2 | 8% | 23 | 92% |
| Neomycin | 25 | 100% | 0 | 0% |
| Kanamycin | 25 | 100% | 0 | 0% |
| Chloramphenicol | 25 | 100% | 0 | 0% |
| Tetracycline | 15 | 60% | 10 | 40% |
| Trimethoprim-sulfamathoxazole | 17 | 68% | 8 | 32% |
| Cephataxime | 25 | 100% | 0 | 0% |
| Cephalexin | 25 | 100% | 0 | 0% |
| Bacitracin | 20 | 80% | 5 | 20% |
| Vancomycin | 25 | 100% | 0 | 0% |
| Erythromycin | 16 | 64% | 9 | 36% |
| Rifampicin | 25 | 100% | 0 | 0% |

n: number of isolates

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 which nearly agrees with records of Ezzeldeen *et al.* [23] who found that 96.6% of *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 isnearly 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 oxacillinin 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 coliforms and fecal coliforms were found in 61chilled samples, the average counts of coliforms in positive samples were $2.26\times10^3$cfu/g in yellow gandoufly, $3.9\times10^3$cfu/g in Black gandoufly and $2.37\times10^3$cfu/g in OM-Elkholoul. The average count of fecal coliforms in positive samples was $1.3\times10^3$cfu/g in yellow gandoufly, $1.22\times10^3$cfu/g in Black gandoufly and $2\times10^3$cfu/g in OM-Elkholoul. These results were nearly obtained previously [3, 40, 41, 42].

Table 5 declares thatthe 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

Table 4: Mean total coliforms and fecal coliforms count among different chilled bivalve molluscus(cfu/g)

| | Black gandoufly | Yellow gandoufly | OM_Elkholoul |
|---|---|---|---|
| Total colifom count | $3.9\times10^3$ | $2.26\times10^3$ | $2.37\times10^3$ |
| Fecal coliform count | $1.22\times10^3$ | $1.3\times10^3$ | $2\times10^3$ |

Table 5: Prevalence of *E. coli* among different bivalve molluscus

| Samples | Number of samples | Positive *E. coli* isolates No | % | Total No |
|---|---|---|---|---|
| Yellow gandoufly | 13 | 0 | 0 | 39.39 |
| Live chilled | 20 | 13 | 65% | |
| Black gandoufly | 13 | 0 | 0 | 36.36 |
| Live chilled | 20 | 12 | 60% | |
| OM_Elkholoul | 13 | 0 | 0 | 32.35 |
| Live chilled | 21 | 11 | 52.3% | |

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, enrofloxacine, ofloxacin, spiramicin and amikacin, while 93.3% of them were sensitive to cephataxime, 90% to cephalexin and amoxy-clavulinic, 83.3% to chloramphenicol, 80% to kanamicin and tetracycline, 76.6% to flumequine, 63.3% to ciprofloxacin, 60% to erythromycin and 50% to pefloxacine, neomycine, 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 lowerpercent 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 6: Antibiotic resistance of E. coli isolates (n=30)

| | Sensitive | | Resistant | |
|---|---|---|---|---|
| Antibiotic | No | % | No | % |
| Ampicillin | 0 | 0% | 30 | 100% |
| Amoxy-clavulinic acid | 27 | 90% | 3 | 10% |
| Amoxicillin | 0 | 0% | 30 | 100% |
| Ofloxacin | 30 | 100% | 0 | 0% |
| Enrofloxacin | 30 | 100% | 0 | 0% |
| Ciprofloxacin | 19 | 63.30% | 11 | 36.60% |
| Pefloxacin | 15 | 50% | 15 | 50% |
| Flumequine | 23 | 76.60% | 7 | 23.3 |
| Gentamicin | 30 | 100% | 0 | 0% |
| Spiramicin | 30 | 100% | 0 | 0% |
| Amikacin | 30 | 100% | 0 | 0% |
| Streptomycin | 0 | 0% | 30 | 100% |
| Neomycin | 15 | 50% | 15 | 50% |
| Kanamycin | 24 | 80% | 6 | 20% |
| Lincomycin | 15 | 50% | 15 | 50% |
| Chloramphenicol | 25 | 83.30% | 5 | 16.60% |
| Tetracycline | 24 | 80% | 6 | 20% |
| Trimethoprim-sulfamathoxazole | 15 | 50% | 15 | 50% |
| Cephataxime | 28 | 93.30% | 2 | 6.60% |
| Cephalexin | 27 | 90% | 3 | 10% |
| Erythromycin | 18 | 60% | 12 | 40% |

n : number of isolates

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* I solates was to cephataxime (79.17%), but Abou-Dobara *et al*. [63] stated that only 26% of the *E. coli* isolates were sensitive to cephotaxime.Moreover 90% of *E. coli* isolates were sensitive to amoxy-clavulinicacid, 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 ciprofloxacin (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.

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