

Faecal Contaminants in Edible Bivalves from Maputo Bay, Mozambique: Seasonal Distribution, Pathogenesis and Antibiotic Resistance

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Abstract: In Maputo, Mozambique marine bivalves considerably contribute to the diet of the population. This study aimed to investigate seasonal distribution of *Escherichia coli* and *Salmonella* in clams from Maputo Bay, and examine their pathogenesis and antibiotic-resistant patterns. Standard multiple tube method revealed that the concentration of coliforms in all samples exceeded the limit for direct consumption, according to EU standards. Thirty-eight percent of the samples contained >60,000 MPN per 100 gram flesh. The occurrence of *E. coli* did not differ significantly due to season, while *Salmonella* was present in 100% of the samples during the rainy period and only in 30% during the dry. Multiplex polymerase chain reaction showed that 45% of *E. coli* isolates were positive for the virulent indicator gene *fimA*. The *Salmonella* isolates were identified as *S. enterica* serovar Typhimurium. Among other isolated coliformic Enterobacteriaceae, *Shigella* sp. (*specie*), which in low doses can cause severe gastrointestinal infections, was identified. Antimicrobial susceptibility, recorded by the disk diffusion method, showed resistance to the most commonly used antibiotics. This high levels of faecal contaminants in the clams points out the need for risk assessment and sanitary improvements.

INTRODUCTION

Marine bivalve mollusks, like oysters, mussels and clams, are globally important food resources. They are particularly important in developing countries; mostly because they are easily collected in shallow areas and have high nutritional value. These species are sedentary and filter-feeding which favor bioaccumulation of microorganisms, which make them frequently involved in outbreaks of gastroenteritis [1-3]. Investigations of shell middens testify that the exploitation of bivalves by man has increased enormously over recent decades in southern Africa [4]. Maputo Bay in southern Mozambique is a sheltered area where people regularly collect clams from the intertidal flats and shellfish contributes considerably to the diet of the population [5]. The water is highly contaminated by shipping and untreated sewage from point and non-point sources of Maputo City and from the rivers entering the Bay. Despite this fact the sanitary safety of shellfish has been neglected, with only a few studies on human enteric pathogens. In 1993 Fernandes *et al.* [6] reported *Salmonella* spp. (*specie plurium*) and *Vibrio* spp. and in 2006, Nenonen *et al.* [7] detected a high prevalence of Hepatitis A in clams from Maputo, indicating that bivalves in the region can be a significant vector of both enteric bacteria and viruses.

In developing countries diarrhea diseases are common among all age groups and constitute one of the main health problems [8]. Beside malaria and tuberculosis, diarrhea is registered as the principal infectious disease in Maputo [9]. In addition, it is the main cause of illness in travelers from industrialized countries to the developing world [10, 11].

Due to poor sanitary conditions there are certainly several emerging sources for transmission of pathogens to humans, such as contaminated drinking water. However, the rapid economic development and population growth have without doubt increased which raise the pollution of the coastal water. Thus the high consumption of bivalves harvested in the Bay might contribute to transmission of pathogens causing gastroenteritis and be of significance for public health.

Escherichia coli is frequently used as an indicator organism for fecal contamination in food and water. This species is in most cases quite harmless but some strains are highly pathogenic to humans. Especially in infants in developing countries the enteropathogenic *E. coli* (EPEC) and the enterotoxigenic *E. coli* (ETEC) are commonly implicated in diarrhea [12, 13]. Contrary to *E. coli*, *Salmonella* spp. are in general pathogenic to humans and represents one of the main causes of food borne illness worldwide [14]. Sea food in particular has been recognized as potential vectors [15-17]. Multi-drug resistance among non-typhoidal *Salmonellae* (NTS) tends to increase in Eastern Africa [18] and if spread in the marine environment it can contribute to health problems. In order to initiate risk assessment for bivalve consumption the aim of this study was to investigate seasonal distribution of *E. coli* and *Salmonella* in clams from Maputo, and examine their pathogenesis and antibiotic-resistant patterns.

MATERIAL AND METHODS

Area Description, Sample Collection and Preparation

Maputo Bay, in southern Mozambique (Fig. 1) is a semi-enclosed bay which opens into the Indian Ocean in the northeast.

The capital, Maputo, with approximately two million residents, is situated on the western side of the bay where the

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rivers Umbeluzi, Tembe and Matola enter the harbor. Another two rivers drain into the bay, the Incomati River in the northern end and Maputo River in the south-west. The highest flows are recorded in these two rivers, which supply the bay with a yearly mean of 200-300 m³ per sec of freshwater [19]. The tide of the area is semi-diurnal with a range at spring of about 3 m and the residence time for the water of the bay versus oceanic water is approximately two weeks. Southern Mozambique belongs to the sub tropical zone characterized by two seasons; a warm and rainy (October-March) and a colder and dry (April-September). In order to investigate seasonality in the occurrence of the pathogens the sampling was carried out in May-June 2004 and in November 2005. Clams of the two most abundant edible species, *Meretrix meretrix* and *Eumarcia paupercula*, were bought from collectors at two popular gathering sites; Bairro dos Pescadores in the north eastern part of the bay and Bairro Luis Cabral in the inner part, close to the harbor. Clams were also bought from three different fish markets along the coastline of the bay (Central market, Maritimo and Costa do Sol). In total 34 samples were collected. Twenty-two of them were dispersed over a period of five weeks during the dry season and 12 samples were dispersed over two weeks during the rainy season. After purchase, the clams were placed in plastic bags and directly transported to the laboratory. Analyses started within one hour. Damaged or open clams were discarded and the remaining clams were opened with a flame sterilized shucking knife. Ten to 20 individuals of *M. meretrix* or *E. paupercula*, given a total weight of 50-70 grams, were used for each analysis.

Total Coliforms and *E. coli*

Clam tissue was homogenized in 2 ml 0.1% peptone buffer (pH 7.2; Merck, Darmstadt, Germany) per gram. Most probable number (MPN per 100 g soft tissue) of coliforms was determined using the standard multiple tube method [20] with 5 replicates; 4 dilutions each, in Mineral Modified Glutamate Broth [1000 ml: 11.4g Minerals Modified Medium Base (Oxoid LTD., Basingstoke, Hampshire, England); 6.4g

Sodium Glutamate (Oxoid), and 5g Ammonium Chloride]. The tubes were incubated at 37±2°C for 24±2 h. One µl from each positive tube was recultivated onto Tryptone Bile Glucuronide Agar (Chromocult® TBX, CM 0945 A; Oxoid) plates and incubated at 44±1°C for 22±2 h. From each sample two to five colonies that were morphologically judged as *E. coli*, were isolated and transported in Liquid Media Transport Swabs tubes (COPAN Italia S.p.a., Brescia Italy) to Kristianstad University, Sweden, for identification, virulence screening and antibiotic resistance tests. API-20E (bioMérieux Inc., Hazelwood, MO) was used for the identification of the *E. coli* isolates. The isolates were added to twenty pre-treated mini-test tubes in accordance to the manufactures' instructions and incubated at 37°C for 20±2 h. The biochemical characteristics were evaluated with the software provided by bioMérieux Inc.

The identified *E. coli* isolates were screened for presence of the virulence genes *iut*, *fimA*, *sfaD*, *papC*, *hlyA*, *eaeA*, *VT1*, *VT2*, *perA*, *LT*, *ST*, *pet*, *astA* and *aggR* (Table 1) using Multiplex PCR [21]. Briefly, 0.5 µl of each of the primers and one bacterial colony were added to 25 µl Master Mix (HotStarTaq Master Mix, Qiagen,) and sterile H₂O was added to adjust the final volume to 50 µl. The PCR amplification was performed according to Table 1. PCR products were run on gel electrophoresis (80V, 30-60 min) using DNA ladder GeneRuler™ 100 bp as size standard and examined under UV-light illumination.

Salmonella spp.

The presence of *Salmonella* spp. was determined by standard method NMKL nr71 from the Nordic Committee on Food Analysis [22]. Homogenized clam tissue (25 g) was pre-enriched in 225 ml 0.1% peptone buffer at 37°C for 18 h. Subsequently 100 µl were transferred to each of five tubes containing pre-warmed Rappaport-Vassiliadis Soy Peptone Broth (CM0866, Oxoid). The tubes were incubated at 41.5±0.5 °C for 24±3 h. One µl of the enrichment was spread onto five replicates of Xylose Lysine Desoxylate Agar

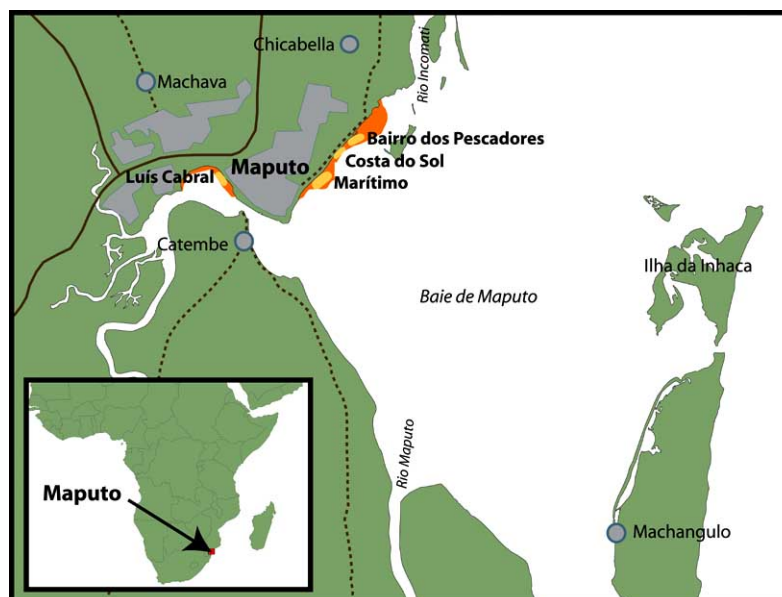


Fig. (1). Map showing Maputo, the capital of Mozambique, as well as the two sampling sites (Bairro dos Pescadores and Bairro Luis Cabral) at the outer and inner part, respectively, of Maputo Bay.

Table 1. List of Primers (in Accordance to Publications in Reference list) that were used for the PCR Analyses of Genes Encoding Different Virulent Factors in *E. coli* Strains Isolated from Clams

| Virulence Factor (biotype) | Gene | Primer Sequence 5'-3' [ref] | Product (bp) | Annealing (°C-sec) |
|--|----------------------------|---|--------------|--------------------|
| Aerobactin | <i>iutA</i> | GGC TGG ACA TCA TGG GAA CTG G CGT CGG GAA CGG GTA GAA TCG [39] | 301 | 65/60 |
| Type 1 fimbriae | <i>FimA</i> | CGA CGC ATC TTC CTC ATT CTT CT ATT GGT TCC GTT ATT CAG GGT TG [21] | 721 | 65/60 |
| <i>S. fimbriae</i> | <i>sfaD</i> <i>sfaE</i> | CTC CGG AGA ACT GGG TGC ATC TTA C CGG AGG AGT AAT TAC AAA CCT GGC A [40] | 410 | 65/60 |
| <i>P. fimbriae</i> | <i>papC</i> | GAC GGC TGT ACT GCA GGG TGT GGC G ATA TCC TTT CTG CAG GGA TGC AAT A [40] | 328 | 65/60 |
| Haemolysin | <i>hlyA</i> | AAC AAG GAT AAG CAC TGT TCT GGC T ACC ATA TAA GCG GTC ATT CCC GTC A [41] | 1177 | 65/60 |
| Attaching and effacing, intimin structural gene (EHEC, EPEC) | <i>eaeA</i> | AAA CAG GTG AAA CTG TTG CC CTC TGC AGA TTA ACC TCT GC [42] | 454 | 57/60 |
| Shiga toxin 1 (EHEC) | <i>VT1</i> | GAA GAG TCC GTG GGA TTA CG AGC GAT GCA GCT ATT AAT AA [43] | 130 | 55/45 |
| Shiga toxin 2 (EHEC) | <i>VT2</i> | ACC GTT TTT CAG ATT TT ^G _A CAC ATA TAC ACA GGA GCA GTT TCA GAC AGT [43] | 298 | 55/45 |
| Plasmid-encoded regulatory region (EPEC) | <i>perA</i> | TGT CAT CCT TAG TGC TTC AT GGC AAT GTT CCT TGT GTA AT [42] | 354 | 57/60 |
| Heat-labile enterotoxin (ETEC) | <i>LT</i> | GCG ACA AAT TAT ACC GTG CT CCG AAT TCT GTT ATA TAT GT [44] | 708 | 56/120 |
| Heat-stable enterotoxin (ETEC) | <i>ST</i> | CTG TAT TGT CTT TTT CAC CT GCA CCC GGT ACA AGC AGG AT [44] | 182 | 56/120 |
| Plasmid-encoded heat-labile toxin (EAggEC) | <i>Pet</i> | TCA TTT CCA GCA CTT CCT GT CTC CGA CAG TAT TTG CTC GT [42] | 442 | 57/60 |
| Heat-stable enterotoxin (EAggEC) | <i>astA</i> | GCC ATC AAC ACA GTA TAT CC GAG TGA CGG CTT TGT AGT CC [42] | 106 | 57/60 |
| Transcriptional activator for aggregative adherence fimbriae I expression (EAggEC) | <i>aggR</i> | GTA TAC ACA AAA GAA GGA AGC ACA GAA TCG TCA GCA TCA GC [45] | 254 | 57/60 |

(XLD; CM0469, Oxoid) and Triple Sugar Iron Agar (TSIA; CM 0277, Oxoid), and incubated at 37°C for 24 h. Isolated *Salmonella* colonies were transported to the Department of Clinic Bacteriology, University of Gothenburg, Sweden (May-June 2004; n=4; full serotyping not performed) and to the Bacteriological Laboratory at the Central Hospital in Kristianstad, Sweden (November 2005; n=21; full serotyping

performed) for identification and antibiotic resistance tests. Putative *Salmonella* colonies were identified by seven tube tests (Indol, H₂S, ODS, Mannitol, Urea, LDC and VP) and phage typing. Both these laboratories use the quality assurance system stated by The Swedish Board for Accreditation and Conformity Assessment.

Antibiotic Resistance

The isolates were tested for antibiotic resistance by the disc diffusion method according to Swedish Reference Group for Antibiotics (SRGA) and its subcommittee on methodology (SRGA-M) (<http://www.srga.org/> Accessed 04-09-01). Single bacterial colonies were diluted in 10 ml sterile 0.9% NaCl and spread onto specific antibiotic resistance plates (CM0471 'ISO-sensitest' agar, Oxoid) and different antibiotic test discs (Oxoid) were applied on the plates. These were incubated at 37°C for about 24 h and the size of inhibition zones was then measured and judged according to the breakpoint values (mm) that give the criteria for antibiotic resistance, intermediate or complete sensitivity.

Statistical Analyses

The comparison between levels of *E. coli* (MPN per 100 g tissue) obtained from the different seasons was analyzed using One-way analysis of variance (ANOVA). Two-way ANOVA was used to compare differences between sites during the two seasons, after balancing the numbers of data by a random exclusion of one of the samples from Bairro Luís Cabral (Sigma Stat, version 3; Jandel Scientific Software, San Rafael, CA). The homogeneity of data and power of analyses were tested before entering the ANOVA. The numbers of virulent genes of *E. coli* that were found during the rainy and dry seasons, respectively, were compared using t-test. Significant value was set to $p < 0.05$.

RESULTS

Total Coliforms and *E. coli*

The mean log-MPN per 100 g tissue of total coliform bacteria analyzed during the dry season (n=22) was 4.68 ± 0.87 and 4.18 ± 0.89 during the rainy season (n=12; $p = 0.161$). The corresponding values of thermo tolerant *E. coli* were 3.63 ± 0.77 and 3.96 ± 0.83 , respectively, indicating no significant differences between seasons ($p = 0.328$). Pooled data from both of the seasons showed no statistically significant differences ($p = 0.097$) of *E. coli* between samples bought from the markets (mean log-MPN per 100 g: 3.43 ± 0.84 ; n=21) and the collectors (mean log-MPN per 100 g: 3.97 ± 0.79 ; n=13). The comparison between the two sampling sites, Bairro dos Pescadores (mean log-MPN per 100 g: 3.43 ± 0.40 ; n=6) and Bairro Luís Cabral (mean log-MPN per 100 g: 4.87 ± 0.42 ; n=6), showed a significant difference (Sum of square 4.43; F 17.8; $p = 0.003$). The two-way ANOVA confirmed that the difference observed between the two sites was not depend on the season (Sum of square 0.56; F 2.25; $p = 0.172$).

According to the Council Directive 91/492 EEC [23] the sanitary level of the collected clams exceeded the level of category A (consumption without restrictions) on every occasion. Category B and C (<6000 and <60,000 MPN coliforms per 100g tissue, respectively) were each represented in 31% of the samples and the rest (38%) were classified as contamination level of "prohibited" (>60,000 MPN coliforms per 100 g tissue). On two occasions there were >500,000 MPN coliforms per 100 g tissue. Other thermo tolerant coliformic species beside *E. coli* that were isolated and identified (n=13) included *Klebsiella pneumoniae pneumoniae*, *K. ornithinolytica*, *K. planticola*, *Enterobacter cloacae* and *Shigella* spp. In addition *Acinetobacter baumannii*, *Flavimonas oryzae*, *Erwinia* spp. and *Serratia marescens* were found. Some samples, especially those from the fish markets, were heavily contaminated with *Pseudomonas* spp.

Fifteen *E. coli* strains were isolated during the rainy season and 60% of these were positive for *fimA*. Fourteen strains were isolated during the dry season and 29% were positive for *fimA* (t-test rainy versus dry season: $p = 0.046$). Altogether 45% of the 29 samples were positive for this virulent gene. None of the isolates were positive for the other gene tested (Table 2).

Salmonella spp.

During the dry season, *Salmonella* spp. were found in three out of 11 samples (one analysis failed) and the bacterial growth was poor, with only a few colony-forming units on each agar plate (Fig. 2). During the rainy season, all samples (n=22) were positive for *Salmonella* spp. and showed dense growth. Samples positive for *Salmonella* spp. were equally distributed among sampling sites. The isolated *Salmonella* strains were identified as *S. enterica* serovar Typhimurium (n=25). Five isolates were further verified to belong to serotype B0, four to C2 and two to C1.

Antibiotic Resistance

The isolated *E. coli* strains (n=29) were sensitive to most of the antibiotics tested except cefadroxil and ampicillin. Forty one % of the strains were resistant to tetracycline (Table 3). The main part of the isolated *Salmonella* strains (n=17) was sensitive to all tested antibiotics apart from cefadroxil, which showed intermediate sensitivity. In addition, one strain was resistant and four were intermediate sensitive to ampicillin.

In general, the resistance among the other coliformic thermo tolerant strains of Enterobacteriaceae (*Klebsiella*

Table 2. List of Reference Strains used for the PCR Analyses of Virulent Genes in *E. coli* Isolates from Clams

| Category | Reference Strains | Target Gene(s) |
|---|--------------------|------------------------------------|
| EHEC | CCUG (DCBS 291 97) | <i>VT1, VT2</i> |
| EPEC | CCUG 36 068 | <i>perA, eaeA</i> |
| ETEC | CCUG 29 197 | <i>LT, ST</i> |
| EAggEC | CCUG 38 083 | <i>aggR, pet, astA</i> |
| <i>E. coli</i> | 233 | <i>iut, FimA, sfaD, papC, hlyA</i> |
| <i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> | CCGU 225 T | Negative control |

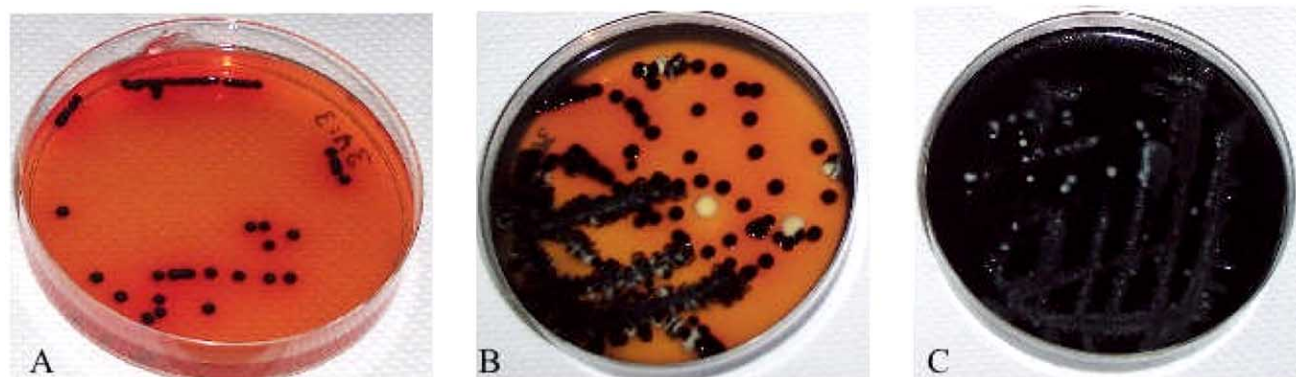


Fig. (2). The images illustrate the seasonal differences in growth density of *Salmonella* colonies on XLD-agar plates. Plate **A** shows the colony density that was representative for the *Salmonella* positive samples during the dry season. Plate **B** and **C** show the high density and black coloring of the agar that were commonly found during the rainy season when 100% of the samples were *Salmonella* positive.

Table 3. Antibiotic Resistance Tests for Isolated Strains of *E. coli*, *Salmonella*, other Coliformic Enterobacteriaceae (*Klebsiella* spp., *E. cloacae* and *Shigella* spp.), and Coliformic Non- Enterobacteriaceae (Others: *A. baumannii*, *F. oryzihabitans*, *Erwinia* spp. and *S. marescens*). The Results are Presented as Numbers of Analysed Strains Judged as S: Sensitivity; I: Intermediate Sensitivity; R: Resistance, respectively

| Antibiotics Tested | <i>E. coli</i> (n=29) | | | <i>Salmonella</i> (n=22) | | | Other Coliformic (n=13) | | | Others (n=7) | | |
|--------------------|-----------------------|-----|-----|--------------------------|----|---|-------------------------|-----|-----|--------------|-----|-----|
| | S | I | R | S | I | R | S | I | R | S | I | R |
| Mecillinam | 27 | 2 | 0 | 22 | 0 | 0 | 11 | 2 | 0 | 2 | 5 | 0 |
| Ampicillin | 0 | 27 | 2 | 17 | 4 | 1 | 0 | 6 | 7 | 0 | 6 | 1 |
| Ceftazidime | 29 | 6 | 0 | 22 | 0 | 0 | 10 | 2 | 1 | 2 | 0 | 5 |
| Cefadroxil | 0 | 24 | 5 | 0 | 22 | 0 | 0 | 11 | 2 | 0 | 2 | 5 |
| Tobramycin | 29 | 0 | 0 | 22 | 0 | 0 | 12 | 0 | 1 | 7 | 0 | 0 |
| Chloramphenicol | 27 | 2 | 0 | 22 | 0 | 0 | 11 | 0 | 2 | 2 | 0 | 5 |
| Ciprofloxacin | 27 | 2 | 0 | 22 | 0 | 0 | 13 | 0 | 0 | 7 | 0 | 0 |
| Norofloxacin | 29 | 0 | 0 | 22 | 0 | 0 | 13 | 0 | 0 | 7 | 0 | 0 |
| Trimethoprim | 29 | 0 | 0 | 22 | 0 | 0 | 10 | 1 | 2 | 3 | 4 | 0 |
| Nitrofurantoin | 29 | 0 | 0 | 22 | 0 | 0 | 8 | 2 | 3 | 2 | 0 | 5 |
| Tetracyclin | 17 | 0 | 12 | 17* | 0 | 0 | 8 | 0 | 5 | 5 | 0 | 2 |
| Gentamycin | 29 | 0 | 0 | 17* | 0 | 0 | 13 | 0 | 0 | 7 | 0 | 0 |
| Nalidixic acid | N.T | N.T | N.T | 17* | 0 | 0 | N.T | N.T | N.T | N.T | N.T | N.T |
| Cephuroxime | N.T | N.T | N.T | 17* | 0 | 0 | N.T | N.T | N.T | N.T | N.T | N.T |
| Trimethoprimsulfa | N.T | N.T | N.T | 17* | 0 | 0 | N.T | N.T | N.T | N.T | N.T | N.T |

*n=17; N.T.: Not tested.

spp., *Enterobacter* spp., *Shigella* spp. *Erwinia* spp. and *S. marescens*) and non-Enterobacteriaceae (*Acinetobacter baumannii* and *Flavomonas oryzihabitans*) were more pronounced. The *Shigella* isolate showed resistance against cefadroxil, chloramphenicol, nitrofurantoin, ceftazidime and tetracycline, and intermediate sensitivity to ampicillin, trimethoprim and mecillinam.

DISCUSSION

This study showed high levels of fecal contamination in bivalves used for consumption in the area of Maputo city,

Mozambique. There is a lack of directive for sanitary safety of bivalves in the country where only exported shellfish such as lobsters are controlled. However, in accordance to the Consul Directive 91/492 [23] that has been practiced within the European Union all samples exceeded the limit for consumption without restrictions (<300 MPN fecal coliforms per 100 g tissue). Almost 40% of the samples fell into the category “prohibited for human consumption” and the rest of the samples were classified as category B or C, which demands depuration before consumption.

The sites chosen for collecting the clams appear to reflect a gradient of contamination, with highest levels of *E. coli* in

the inner part of the bay where the population of Maputo is more concentrated. In contrast to *Salmonella* the occurrence of *E. coli* was seemingly independent of season. Our results showed 100% prevalence of *Salmonella* in the analyzed clam samples from the rainy season but only in 30% of the samples from the dry season. Although the analyses of *Salmonella* were not quantitative, it was noticeable by comparing the growth density after enrichment that the clams contained higher numbers of this bacterium during the warmer, rainy season. This was in agreement with what has been reported by Haley *et al.* [24] from southern Georgia, USA. They found that the increase of *Salmonella* in aquatic environment coincided with both higher temperatures and the rainy season. Another study, committed on more than 5000 *Salmonella* strains [25] isolated from live bivalves in Spain, identified 15% as *S. enterica* serovar Typhimurium. In contrast to the other strains these were found only during warmer periods.

The mean value of precipitation in Maputo during November 2005 was 76.6 mm but only 18.5 mm in May and 24.6 mm in June 2004 (data obtained from The National Institute of Metrology, Maputo, Mozambique). Most probably the transport of fecal contaminants to seawater increases during events of high land run-off [26, 27]. Such events may also increase the turbidity of the shallow Maputo Bay which probably inhibits the bacterial inactivating mechanism of solar radiation as has been shown in studies performed in other areas [28, 29]. The mean water temperature of Maputo Bay rises from 19-20°C during the dry season to 26-28°C during the rainy season [19]. A previous study by [30] showed that growth of virulent strains of *S. enterica* serovar Typhimurium, inoculated in boreal adapted blue mussels (*Mytilus edulis*), were favored by water temperature of 20°C compared to that of 6°C. At the higher temperature the bacteria multiplied with lethal effects on the mussels. Temperature should also be considered during storage of the clams at the fish markets in Maputo where no cooling facilities are available. *Pseudomonas* spp. was often detected in clams from these markets, indicating spoilage of the clam tissue. Beside the effects of the quality of taste, smell and dietary value, high temperature storage may also increase the numbers of pathogenic bacteria. Other environmental properties related to the rainy seasons, like increased level of dissolved organic nutrients and decrease in salinity, may also favor the viability of the bacteria.

Clinical tests have shown an increase of *E. coli* isolates from hospitalized children during the rainy season, which indicates higher virulence of *E. coli* [31]. Also in clams the virulence of *E. coli* was seemingly more pronounced during the rainy season when twice as many of the isolates were positive for the *fimA* gene compared to isolates from the dry season. This gene product is a virulence indicator as it is essential for *E. coli* adherence to intestinal epithelial cells. On the other hand, the positive isolates were negative for other tested virulence genes which indicates that they are not able to cause hemolytic diarrheal disease. Among the other isolated coliformic Enterobacteriaceae, *Shigella* sp. was identified even though we did not use any selective methodology [32] to trace it. This observation is of great concern since *Shigella* even in low doses can cause severe gastrointestinal infection through seafood consumption [33] and it points out the need for further investigations. This is also the

case for the highly virulent *S. typhi*, which may occur in clams but might have been overlooked due to the methodology used in this study. Nevertheless, the frequent findings of *S. enterica* serovar Typhimurium should be considered since it constitute a threat for public health as it is known to cause non-typhoid prolonged gastroenteritis, particularly common among the pediatric population [34].

In general, the antibiotic resistance found in this study was not as pronounced as reported from Europe [35]. However, strains of both *E. coli* and *Salmonella* showed rather prominent resistance against the β -lactams, cefadroxil and ampicillin. This pattern is alarming due to the fact that ampicillin is the main treatment of infectious diarrhea for children under two years of age in rural districts of the Maputo region [31]. Less than 30% of the total isolates from clams were sensitive to ampicillin. Tetracycline was also among the notable antibiotics. Several of the isolated *E. coli* strains (41%) as well as the *Shigella* isolate showed resistance to this inexpensive and widely distributed antibiotic. Resistance to tetracycline has been recognized in many pathogens and commensal bacteria in developed countries and this was also experienced during previous cholera outbreaks in Mozambique, South Africa and Madagascar [36, 37]. According to registration protocols for utilization of antibiotics, kindly provided by The Section of Laboratory, Health Ministry, Maputo, tetracycline is one of the most common antibiotic treatments for human infections. Another antibiotic within this group, the oxitertracycline, is also commonly distributed as used by farmers in the Maputo district for treatment of rickettsia-infected cattle (Pers. Comm. Dr. L. Neves, The Veterinary Institute, Maputo, Mozambique). The antimicrobials most widely regarded as optimal for treatment of salmonellosis in adults are fluoroquinolones [38] and it was satisfying that our results, in agreement with a study of *Salmonella* from children with bacteraemia in Kenya [34], showed high sensitivity to ciprofloxacin. Antibiotic resistance may comprise an increasing threat as these drugs are frequently misused and unregulated available in pharmacies or general stores in Maputo.

CONCLUSIONS

Most of the strains of *E. coli* that were found in the clams from Maputo were seemingly quite harmless. However, the high numbers of coliforms certainly indicated a local and continuous source of fecal contamination with hazardous potential, such as the high prevalence of *Salmonella* and the observed multi-resistant *Shigella*. There might also be more distant sources of pathogens like the recent introduction of more intense livestock in catchments upstream the rivers that enter Maputo Bay. Such a contribution of enteric bacteria may also increase the spreading of antibiotic resistance since antibiotics are commonly used for infection treatments of cattle.

When pathogens accumulate in clams they constitute a particular risk since clams are consumed only lightly cooked to retain the palatable texture and nutritional value. Indeed the results of this initial study indicated a potential risk of consuming clams from Maputo Bay but further studies are needed in order to predict seasonal peaks or epidemic outbreaks of enteric infections, and thereby offer opportunities for mitigation, and even prevention of these kinds of dis-

eases. This is of increasing importance for the growing population of the area, particularly children and for the increasing numbers of immunocompromised individuals, like HIV-infected persons, who are more susceptible to infections. It is also of great importance for the growing tourism which can be of major economic importance.

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