Prevalence of Extended Spectrum Beta Lactamase (ESBL) Producing Escherichia coli and Klebsiella pneumoniae Isolated from Raw Milk Samples in Al Jazirah State, Sudan

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Abstract

Milk play a major role in human sources of nutrition and remain as the most important prominent in the Sudanese diet. Escherichia coli and Klebsiella pneumoniae are humans and animals opportunistic pathogens, responsible for a wide range of infections. The aim of this study was to evaluate the quality of the commercial available milk and to detect ESBL producing E. coli and K. pneumoniae from raw milk samples of cow in Al Jazirah state, Sudan. Seventy fresh raw cow milk samples were collected and examined using standard microbiological methods, ESBL detection was performed on all the isolates by Ceftazidime screening test, those shows positive results by screening method were subjected to ESBL confirmatory test using Double-Disk Synergy Test and Molecular base detection using conventional PCR. Out of the 70 collected samples, 58 (82.8%) showed positive isolating result, the highest prevalence of the isolates was K. pneumoniae 36 (62%) followed by E. coli 22 (38%). The most resistance antibiotics against isolates was Ampicillin (98%), ESBL production was detected among 17 out of the 22 isolated E. coli (29.3%) and 26 (44.8%) out of the 36 isolated K. pneumoniae. The ESBL gene encoding the ESBL isolates was CTX-M gene representing 61% fellows by SHV gene (23%) and TEM gene (16%). ESBL-producing bacteria may also be transferred via waste milk to calves, thus further spreading antibiotic resistance in the farm environment.

Keywords: E. coli, K. pneumonia, Raw milk; ESBL; Al Jazirah State; Sudan

Introduction

Milk is a major part of human food and plays a prominent role in the Sudanese diet.

It's considered as nature's single most complete food; Moreover, its high nutritive value makes it an ideal medium for the rapid multiplication of bacteria, particularly under unhygienic production, production and storage at ambient temperatures [1,2]. Microorganism in raw milk can originate from different sources such as air, milking equipment, feed, soil and grass [3,4]. Largely depends on fecal contamination and the presence of pathogen in feces mainly originates from feed contamination. The presence of pathogenic bacteria in milk is of considerable public health concern, especially for those individuals who still drink raw milk [5]. Enterobacteriaceae are the significant causes of serious infection. Escherichia coli and Klebsiella pneumoniae, an opportunistic pathogen of humans and animals responsible for a wide range of infections, such as diarrhea, urinary tract infections, pneumoniae, wound infections, septicaemia, haemolytic uraemic syndrome and nosocomial infections especially meningitis in infants [6,7]. The appearance of ESBL stated in the 1980s and widely distributed in the world [8,9] and conferred increased resistance to beta lactams except carbapenems and cephamycins [10,11]. ESBLs are plasmid mediated and the genes encoding these enzymes are easily transferable among different bacteria [12]. Most of these plasmids not only contain DNA encoding ESBLs but also carry genes conferring resistance to several non-β-lactam antibiotics [13]. ESBL can hydrolyse penicillins first, second and third-generation cephalosporins and aztreonam (but not cephamycins or carbapenems). Resistance to beta lactam antibiotics is most commonly found in E. coli and K. pneumoniae and today, this resistance mechanism is recognized globally, in the past few years, there has been an increase in the detection of ESBL-producing strains in the general community [14,15]. The antibiotic resistance leads to increased morbidity, mortality and the cost of treating infections, in particular, those caused by ESBL producing bacteria [16]. Microbiological assessments have an important role to play in the dairy industry to protect the public health and can reduce economic losses. The objective of this study was to isolate and identify E. coli and K. pneumoniae from raw milk samples of cow and to evaluate the antibiotic sensitivity pattern.

Materials and Methods

Study area and sampling

This was a health facility based cross-sectional study, performed from March to August 2017. A Total of 70 raw cow milk samples were collected from different villages in Al Jazirah State-Sudan, all samples were collected aseptically, transported to the laboratory under chilled conditions and processed for microbiological analysis.
Isolation and identification of bacteria from raw milk samples

The samples were inoculated into MacConkey’s broth tubes (HiMedia, Mumbai, India) and incubated at 370°C for 18-24 h. A loopful inoculum from MacConkey’s broth was streaked onto Eosin Methylene Blue (EMB) agar (HiMedia, Mumbai, India) and MacConkey’s agar; plates were incubated at 370°C for 18-24 h. After that separation of pure colonies were take place by seeding it onto sterile nutrient agar slants as pure culture and subjected for standard morphological and biochemical tests as well as PCR [17].

Antimicrobial susceptibility testing and ESBL detection

The antimicrobial susceptibility testing of all identified isolates were done according to the criteria of the Clinical and Laboratory Standards Institute method (CLSI). All isolates were screened for ESBL production by using Cefotaxime (CTX 30 μg).

Cefazidime (CAZ 30 μg) and Ceftriaxone (CRO 30 μg). Each isolates which showed resistant to one or more of these antibiotics were confirmed for ESBL production by Double Disk Synergy Test (DDST) recommended by the CLSI guidelines [18].

Molecular detection

DNA for molecular detection was extracted after bacterial lysis according to the extraction protocol prepared by the Community Reference Laboratory for Antimicrobial Resistance, 2009. Briefly, a few colonies taken from fresh culture medium and transferred to phosphate buffered saline (pH 7.3). The suspension was heated at 100°C for 30 min. Boiled suspension was transferred directly on ice. The suspension was then centrifuged at 12,000 rpm for 30 min and the supernatant containing DNA was transferred to new Eppendorf tubes. PCR method was used for resistance encoding genes detection (Table 1). PCR Master mix components, Dream Taq Green PCR Master Mix, Nuclease free water and DNA marker “Gene Ruler” were provided by Thermo Scientific (Lithuania). PCR protocol was described by Community Reference Laboratory for Antimicrobial Resistance, 2009.

Results

A Total of 70 raw cow milk samples were collected from different villages in Aljazira state. Out of them, 58 (82.8%) showed positive isolating result, after morphological and biochemical identified, the highest observed prevalence of the isolates was *K. pneumoniae* (62%) followed by *E. coli* (38%) (Table 2).

### Table 1: Primers used for PCR protocols.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (50–30 bp)</th>
<th>PCR product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTX-MF</td>
<td>ATGGCAGYACAGTAARGT</td>
<td>593</td>
</tr>
<tr>
<td>CTX-MR</td>
<td>TGGGTRAARTGTSSACAGA</td>
<td></td>
</tr>
<tr>
<td>blaSHV-F</td>
<td>CAAAAGCGCGGTTATTC</td>
<td>937</td>
</tr>
<tr>
<td>blaSHV-R</td>
<td>TTACGTTGGCGAGTGCT</td>
<td></td>
</tr>
<tr>
<td>blaTEM-F</td>
<td>GAGTATCCACATTCTCGT</td>
<td>857</td>
</tr>
<tr>
<td>blaTEM-R</td>
<td>ACCAATGCATTACAGTA</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Prevalence of organism contaminating row cow milk.

<table>
<thead>
<tr>
<th>Isolated Microorganisms</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>36</td>
<td>62</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>58</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

### Table 3: Antibiotic resistance against isolated *E. coli* and *K. pneumoniae*.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>% Resistance among bacterial isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>98</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>91</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>67.5</td>
</tr>
<tr>
<td>Amikacin</td>
<td>63.2</td>
</tr>
<tr>
<td>Cefepime</td>
<td>95</td>
</tr>
<tr>
<td>Imipenem</td>
<td>19.9</td>
</tr>
</tbody>
</table>

### Table 4: Distribution of ESBL strains according to screening and confirmation test.

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive ESBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli N (%)</td>
<td>K. pneumoniae N (%)</td>
</tr>
<tr>
<td>ESBL screening</td>
<td>20 (90.9)</td>
</tr>
<tr>
<td>ESBL DDST</td>
<td>17 (47.2)</td>
</tr>
<tr>
<td>PCR</td>
<td>16 (40)</td>
</tr>
</tbody>
</table>

Discussion

In this study, we screened for ESBL-producing *E. coli* and *K. pneumoniae* from raw-cow-milk sample. The results highlighted that 52 isolates were positive for ESBL-producing. ESBL producing *E. coli* and *K. pneumoniae* isolates were confirmed by Double Disk Synergy Test (DDST).

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coli observed resistance of consistent with those obtained in previous studies [33,34]. 

Klebsiella the utensils could be the source of accelerating the bacterial surveillance and judicious use of these antibiotics.

worker, poor quality of milk, unhygienic conditions of manufacturing contamination of milk products and the post manufacturing unit, inferior quality of material used and water supplied for washing 1043-1048.

References


However, both milk and milk products the methods of production, handling, transportation and marketing of these products are entirely depend upon traditional system. Such system could pose favorable environment for bacterial contamination. The unclean hands of worker, poor quality of milk, unhygienic conditions of manufacturing unit, inferior quality of material used and water supplied for washing the utensils could be the source of accelerating the bacterial contamination of milk products and the post manufacturing contamination. [47-51]. However, there remains a need for continued surveillance and judicious use of these antibiotics. The increasing prevalence of resistance in the isolates from animal origin may have important therapeutic implications. Thus, monitoring of ESBL-producing enter bacteria should be continued at various level (animals, human and environment), should be reconsidered because it does not only contribute to the spread of pathogenic bacteria but also is a vehicle to spread antibiotic resistance. While investigating the factors that contribute to their selection and dissemination.

Conclusion

The results obtained in this study concluded that the milk available for consumer have a high bacterial contamination. Thus, the results of the present study warn the need for stricter preventive measures. For this, regular sterilization of dairy equipment, washing of utensils, milker’s hands, udders, eradication of diseased animals, pasteurization/boiling of milk is required before collection and distribution for consumption and product making. The magnitude of the problem of bacterial contamination deserves more elaborative studies from the point of production of milk and milk products to the point of consumption and at all intermediary levels. Thus present study suggests isolating and characterizing the E. coli and Klebsiella spp. which may cause the pathogenicity in milk products.

Acknowledgement

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