Detection of bacterial contamination in adult and infant powdered milk consumed in Khartoum State

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Abstract:

This study was carried out to evaluate the microbiological contamination of powdered milk, The prevalence of pathogenic bacteria and antimicrobial susceptibility patterns, in a total of 60 samples of commercial dried milk products (adult powder milk and infant powdered milk 30 samples each) used by consumers in Khartoum state (Sudan), conventional culture method were used for isolation and identification. The total viable bacterial count of adults powdered milk ranged between 1-38 (CFU)/ml, and the total viable bacterial count for infants ranging from 0-28 (CFU)/ml. The total number of different species of bacteria isolated from adult powdered milk samples was 45; Bacillus cereus isolates were 17 (37%), Bacillus leichiniformis were 11 (24%), Bacillus mycoides were 3 (7%). Staphylococcus aureus 9 (20%), Listeria monocytogenes 5 (11%). The total number of different species of bacteria isolated from infant formula samples was 39; Bacillus cereus isolates were 12 (30%), Bacillus leichiniformis 7 (18%), Bacillus mycoides 2 about (5%). Staphylococcus aureus 2 (5%), Listeria monocytogenes 3 (7.9%), Enterobacter sakazakii 9 (23%), Escherichia coli 4 (9%). In relation to antibiotic resistance of bacteria isolated from both infant formula and adult’s powdered milk. Staphylococcus aureus resistance against Amoxycillin and Tetracycline was between (45-60%), Erythromycin (30-40%), Ceftriaxone Ciprofloxacin (25-30%) and Cefixime was (20-35%). Bacillus species resistance against tetracycline was (30-50%) , against Amoxycillin, Ceftriaxone, Ciprofloxacin was (20-30%) and Cefixime was (20-35%). Listeria monocytogenes resistance against tetracycline was (40%), Amoxycillin (25-30%), Ciprofloxacin (12-25%), Cefixime (20%), Ceftriaxone (15-20%), Erythromycin (10-20 %). Enterobacter sakazaki resistance against Amoxycillin was (50%), tetracycline (40%), Ciprofloxacin, Cefixime, Ceftriaxone (15-20%). The lowest resistance of all isolates was seen against Amikacin and Meropenem (0-5%).

Introduction:

Milk and milk products have been familiar foods since the old days, and the volume of production is increasing with the passage of time. Recently, the functions of useful trace components in milk have attracted attention (Tomita,2000). There is a dearth of information on contamination of powdered milk in developing countries, and there has also been no surveillance on the disease resulting from consumption of contaminated powdered milk (FAO/WHO, 2004, 2006). The World Health Organization (WHO) recently expressed concern regarding the safe handling, preparation and delivery of powdered infant feeds (PIFs) in health care settings. PIFs are not sterile and may contain pathogens such as
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Salmonella, Enterobacter sakazakii (Cronobacter) and other enterobacteriaceae. PIFs have been associated with serious illness and death in some infants. (FAO/WHO, 2004) (Marino et al, 2007). Pathogen detection is a critical parameter linked to the safety of powdered infant formula (PIF). Because PIF effectively supports the growth of numerous pathogens, it can become easily contaminated (Min Wang et al-2009). In most studies reviewed, contamination of infant food formula by pathogenic microorganisms at some points during production resulted in several outbreaks of diseases worldwide. The use of infant formula in developing countries has caused higher rates of diarrhoeal morbidity and mortality, possibly because contaminated water is often used to prepare infant formula and because the high nutrient contents of infant formula provide a good growth medium for bacterial pathogens (Shadlia-Matug et al, 2008). Dried milk products and infant food are known to be frequently contaminated with Bacillus cereus (Becker et al, 1994). Cronobacter is a major foodborne pathogen in powdered infant formula and can lead to serious developmental after-effect and death to infants. The contamination of Cronobacter may be a high risk for the powdered foods (Van Acker et al, 2001). Cronobacter is distributed and frequently contaminated in the environment, in particular, contamination on powdered infant formula occurs more easily because it is a nonsterilized product. Contamination is caused by poor handling and added raw ingredients during processing, drying, and packaging (Lee et al, 2010). Nazarowec-White and Farber (1997) reported that E. sakazakii was more thermo tolerant than most other Enterobacteriaceae which may contribute to its survival of heat treatments, and subsequent presence in desiccated products.

The world wide increase of food borne infections with antibiotic resistant pathogens is of growing concern and is designated by the WHO as an emerging public health problem (Ledergerber et al, 2003) (Teuber, 1999). Nutritive and therapeutic treatment of farm animals with antibiotics, amounting to half of the world's antibiotic output, has selected for resistant bacteria that may contaminate the food produced. Antibiotic-resistant enterococci and staphylococci from animals are found in food when they survive the production processes, as in raw cured sausages and raw milk cheeses. The broad host ranges of some plasmids and the action of transposons in many bacteria allow antibiotic-resistance genes to be communicated by conjugation between different species and genera (Vincent Perreten et al, 1997).

Material and methods:
Mainly, milk powder used by adults and infants, were collected from supermarkets and pharmacies, 60 samples (30 samples each).
Sampling methods of infant formula milk and powdered milk:

Twenty five grams of powdered milk weighted, dissolved in 225ml of pre warmed sterile peptone water at 40°C, and incubated for 15-20 min in a water bath at the same temperature, serial dilutions were prepared. Using the method described by Roberts et al. (2004). For salmonella, 10 ml from the sample were inoculated in 90 ml sterilized Selenite-F broth using sterile pipettes, incubated at 37° C for 24 hours, then 0.5 ml were poured on XLD agar, spread using a sterile glass spreader rod. For aerobic plate count (APC), 0.5 ml were poured on plate count agar using microtitre pipette and spread with a sterile glass rod and incubated at 37° C for 24 hours. Visible colonies were counted using Quebec Colony Counter. 0.5 ml were poured on Mannitol salt agar, MacConkey agar, Blood agar and E. M. B. agar and spread using sterile glass rod and incubated at 37° C for 24 hours (Muytjens et al,1988).

Identification of bacteria:

Bacteria were identified as follows: The resulting growth was checked for purity by staining with Gram's stain and examined microscopically. Then all the isolates were subjected to biochemical tests, which were performed according to Barrow and Feltham (2003), and Cheesbrough (2000).

Susceptibility of isolated bacteria to different antibiotics:

Sensitivity of the different isolates to 8 antibiotics was studied by using Standard disc diffusion method (Amikacin, amoxicillin, Erythromycin cefixime, ceftriaxone, ciprofloxacin, tetracycline and meropenem). All results were recorded appropriately and interpreted using the National Committee for Clinical Laboratory Standards (NCCLS) interpretation chart (NCCLS, 2002).

Results:

The total viable bacterial count of adults powdered milk ranged between 1-38 Colony Forming Unit (CFU)/ml, and the total viable bacterial count of infant formula ranging from 0-28 (CFU)/ ml.

Numbers and types of bacteria isolated from adult’s powdered milk:

The total number of different species of bacteria isolated was 45; the numbers of Bacillus cereus in adult’s powdered milk samples isolates was 17 (37% ), Bacillus leichiniformis was 11 (24% ), Bacillus mycoides was 3 about (7%). Staphylococcus aureus was 9 (20%), Listeria monocytogenes was 5 (%11) (table 1).

Numbers and types of bacteria isolated from infant formula:

The total number of different species of bacteria isolated from infant formula samples was 39; the numbers of Bacillus cereus isolates was fairly high 12 (30%), Bacillus leichiniformis was 7 (18%), Bacillus mycoides was 2 about...
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Mohammed et al. (5%). *Staphylococcus aureus* was 2 (5%), *Listeria monocytogenes* was 3 (7.9%), *Enterobacter sakazakii* was 9 (23%), *Escherichia coli* 4 (9%) (table 2).

**Antibiotic susceptibility patterns of the isolates**

The susceptibility patterns of all bacterial isolates against various antibiotics are shown in (Tables 3 and 4)

Table (1): Species isolated from 30 samples of infant formula

<table>
<thead>
<tr>
<th>No.</th>
<th>Species isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>17</td>
<td><em>Bacillus cereus</em></td>
</tr>
<tr>
<td>11</td>
<td><em>Bacillus leichiniformis</em></td>
</tr>
<tr>
<td>3</td>
<td><em>Bacillus mycoides</em></td>
</tr>
<tr>
<td>5</td>
<td><em>Listeria monocytogenes</em></td>
</tr>
<tr>
<td>45</td>
<td>Total</td>
</tr>
</tbody>
</table>

Table (2): Species isolated from 30 samples of adult powdered milk

<table>
<thead>
<tr>
<th>No.</th>
<th>Species isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>12</td>
<td><em>Bacillus cereus</em></td>
</tr>
<tr>
<td>7</td>
<td><em>Bacillus leichiniformis</em></td>
</tr>
<tr>
<td>2</td>
<td><em>Bacillus mycoides</em></td>
</tr>
<tr>
<td>3</td>
<td><em>Listeria monocytogenes</em></td>
</tr>
<tr>
<td>9</td>
<td><em>Enterobacter sakazaki</em> (Cronobacter)</td>
</tr>
<tr>
<td>4</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>39</td>
<td>Total</td>
</tr>
</tbody>
</table>

Table (3) resistance percentage of adult samples isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resist % <em>S.aureus</em></th>
<th>Resist % <em>Bacillus spp.</em></th>
<th>Resist % <em>Listeria monocytogenes</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>40</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Cefixime</td>
<td>35</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>30</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>25</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>60</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>45</td>
<td>30</td>
<td>25</td>
</tr>
</tbody>
</table>
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Table (4) resistance percentage of infant formula isolated species

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resist % S.aureus</th>
<th>Resist % Bacillus spp.</th>
<th>Resist % Listeria monocytogenes</th>
<th>Resist % E. sakazaki</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>50</td>
<td>25</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>Cefixime</td>
<td>20</td>
<td>25</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>25</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>25</td>
<td>27</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>50</td>
<td>30</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Erythromycine</td>
<td>30</td>
<td>20</td>
<td>10</td>
<td>-</td>
</tr>
</tbody>
</table>

Discussion:

Pathogen detection is a critical parameter linked to the safety of powdered infant formula (PIF). Because PIF effectively supports the growth of numerous pathogens, it can become easily contaminated. Various studies examining PIF contamination have identified various pathogenic bacteria (Min Wang et al.-2009).

In the present study powdered milk (adults and infant formula) samples were investigated for the presence of bacterial contaminants. The results showed that some of the milk powder types contain different species of bacteria namely Bacillus species, Enterobacter sakazakii, staphylococcus aureus, Listeria monocytogenes and E. coli. Bacillus spp isolates were a reflection to their wide spread and survival in the environment due to their ability to form resistant endospores. They include B. cereus, B. licheniformis and B. mycoides. Similar findings were reported by Vaisanen et al., (1991) who isolated Bacillus strains from various dairy products. Ahmed et al., (1983) stated that Bacilli, especially members of the Bacillus Cereus group, are common contaminant of milk and dairy products, as well as products based on dried milk. In this study 76.9% of Bacillus cereus were isolated from (adults) powdered milk and 44.4% from (infant) powdered milk. This finding is in agreement with those of Reves et al.,(2007) who isolated this species from dried milk. Bacillus mycoides isolated in this investigation constituted (23.1%) from adult’s and (11.11%) from infant powdered milk. This species was shown to be capable of producing diarrhoeagenic enterotoxin (Griffiths, 1990). Bacillus licheniformes was also isolated (61.5%) from adults and (38.8%) from infant samples.
infant powdered milk and it was regarded as health threatening as stated by Andersson et al., (1998). The *E.coli* bacteria are known to be a predominant cause of diarrhea in developing countries that contamination of milk with *E.coli* is unacceptable in other parts of the world and indicates fecal pollution and lack of sanitary quality. (Jay, 1986) (Morais, 1995). In this study *E.coli* (22.2%) was isolated from infant’s formula samples only, which may be due to contamination during processing. The use of infant formula in developing countries has caused higher rates of diarrhoeal morbidity and mortality, possibly because contaminated water is often used to prepare infant formula and because the high nutrient contents of infant formula provide a good growth medium for bacterial pathogens (Fone et al., 2002).

*E. sakazakii* is an emerging pathogen, often transmitted through the consumption of powdered infant foods and its products. It is responsible for series of infections with potential fatal outcomes in infants. The *Enterobacter sakazakii* isolated in this study is in agreement with the results of studies carried out in New Zealand, 1986, 1991 and 2004 where four cases have been linked to consumption of infant formula causing infection in premature babies, supported by (International Baby Food Action Network IBFAN) and other studies reported that *Enterobacter sakazakii* causes major infections especially among neonates (Nazarowec and Farber, 1997) (Lai, 2001).

In this study *Listeria monocytogenes* was isolated from (11.11%) of infant milk samples tested and (38.5 %) of adults samples. This may be due to their tolerance to the preparation process. *Staphylococcus aureus* was isolated from about (69.2%) of adult’s samples and about(11.11%) of infant milk samples. *S.aureus* is a contaminant of milk and dairy products as it is a major cause of mastitis in dairy cattle .This result agree with the results obtained by Payne and wood,(1974) ,Pereira et al.,(1991) and Connell (2002). The presence of *S. aureus* strains in powdered milk poses a potential threat to human health.

In relation to antibiotic resistance of bacteria isolated from both infant formula and adult’s powdered milk. *Staphylococcus aureus* resistance against Amoxycillin and Tetracycline was between (45-60%) each, Erythromycin 30-40% each, Ceftriaxone Ciprofloxacin (25-30%) each Cefixime (20-35%), the lowest resistance was seen against Amikacin ,Meropenem (0-5%). Wang X et al (2012) in their study in china found that *Staphylococcus aureus* resistance was most frequently observed to erythromycin (75.9%), followed by ciprofloxacin (51.9%) , tetracycline (18.5%), or cefoxitin (3.7%).

*Bacillus species* resistance against antibiotics tested was as follows, tetracycline (30-50%), Amoxycillin and Ceftriaxone (20-30%) and Erythromycin, Ciprofloxacin, Cefixime (20-27%). All the isolates were (100%) sensitive to Amikacin and Meropenem. Shadlia-Matug et al., (2008) in their study found that *Bacillus* spp showed the highest level of resistance to cephalosporin (36.4%), penicillin (18.1%). Corresponding values for *Staphylococcus* spp were, erythromycin 30%, penicillin 30%, cephalosporin 10%, respectively.

*Listeria monocytogenes* resistance against tetracycline was (40%), Amoxycillin (25-30%), Ciprofloxacin (12-25%), Cefixime (20%), Ceftriaxone (15-20%), Erythromycin.
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Antimicrobial resistance genes present in foodstuffs, either contained in bacteria and bacteriophages or as DNA fragments, may involve an indirect risk for public health as they increase the gene pool from which (pathogenic) bacteria can pick up antimicrobial resistance genes and possibly transfer them to other (pathogenic) bacteria (Claire Verraes et al., 2013).

Conclusion:
Our findings indicate that adult and infant powdered milk used by consumers in Khartoum state were contaminated with Bacillus cereus, Bacillus leichiformis, Bacillus mycoides, Staphylococcus aureus, Listeria monocytogenes, Enterobacter sakazakii and Escherichia coli. In addition these isolates exhibited multiple antimicrobial resistance. Antimicrobial resistant pathogens present on food constitute a direct risk to public health.

Bacillus cereus isolates were 17 (37%), Bacillus leichinformis were 11 (24%), Bacillus mycoides were 3 (7%), Staphylococcus aureus 9 (20%), Listeria monocytogenes 5 (11%).

The number of the different species of the bacteria isolated from food samples is 39.

Bacillus cereus isolates were 12 (30%), Bacillus leichinformis 7 (18%), Bacillus mycoides 2 about (5%), Staphylococcus aureus 2 (5%), Listeria monocytogenes 3 (7.9%), Enterobacter sakazakii 9 (23%), Escherichia coli 4 (9%).
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