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Letter to the Editor

Shigella in baby bottles of a Brazilian newborn nursery

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Dear Editor,

Cronobacter spp. (formerly *Enterobacter sakazakii* as described) and *Salmonella enterica* are the most worrisome pathogens associated with infant formula, since there is clear evidence that their presence can result in severe disease in at-risk population who consume that food [1]. Many studies report the survival of *Cronobacter*spp. in infant milk formula (IMF), but a small number studies reported the detection of *Salmonella* or other Gram-negative bacteria [1,2].

Our work initially aimed at detecting the presence of *Salmonella* spp. in samples of utensils (baby bottles, spoons, teats, jars and trays) and reconstituted IMF from a infant formula preparation room (called lactary in Brazil) in the newborn nursery of a public hospital located in Rio de Janeiro, Brazil. None of the samples studied in this work yielded *Salmonella*, but further identification of the 44 colonies growing in selective media used for the detection of this pathogen revealed the occurrence of other Enterobacteriaceae species in IMF and utensils, as *Acinetobacter baumannii-calcoaceticus* and *Enterobacter cloacae*. However, the most surprising finding was the presence of five (11.4%) isolates obtained from baby bottles identified as *Shigella dysenteriae*type 1, the most toxic of serotypes associated with shigellosis, an acute enteric infection [3].

Although not expected, the presence of this pathogenin baby bottles was not an isolated incident, since the studies by Fauziah *et al*. [4] showed the presence of *Shigella*s pp. in infant formulas prepared in nurseries of neonatal intensive care units.

Foodborne infections caused by *Shigella* and some *Salmonella* species are responsible for millions of illnesses each year and infants are particularly susceptible to these organisms [5,6]. According to Jain and colleagues [3], shigellosis is still an important public health problem in developing and under-developed countries and the ingestion of *Shigella dysenteriae* in infant formulas can cause potentially devastating consequences to newborns. Baby bottles can be considered a potential route of *Shigella*s pp. transmission and a significant factor in the epidemiology of shigellosis, since poor sanitation and inadequate hygiene practices may facilitate the spread of enteric pathogens [4,7,8].

In a lactary, *Shigella* may easily spread to infants fed with IMF and antimicrobial treatment is required for the control of outbreaks [9]. Early use of antibiotics, within the first 72 hours of illness, can limit the clinical course of the disease and the duration of fecal excretion of the pathogen. However, there is a high proportion of *Shigella* resistant to sulfamethoxazole and trimethoprim, which deserves attention because they are the drugs of choice to treat patients with inflammatory diarrhoea [5,10]. In this study, to evaluate the antimicrobial resistance, the *Shigella* isolates were submitted to antibiotic susceptibility testing, according to the Clinical and Laboratory Standards Institute guidelines [11]. All five isolates were resistant to trimethoprim and four of them - ME1, ME3, ME4 and ME5 - presented a typical profile of multi-drug resistance, since they were resistant to antibiotics belonging to at least 3 different classes [12], which is a worrying fact. Results are presented in Table 1.

Table 1. Characteristics of the *Shigelladysenteriae* isolates studied in this experiment.

Isolates	Source	Resistance profile	Biofilm production detection
ME1	baby bottle	Amc, Amp, Atm, Cfl, Ctx, Clo, Ipm, Tri	-
ME3	baby bottle	Amc, Amp, Atm, Cfl, Ctx, Clo, Ipm, Tri	-
ME4	baby bottle	Amc, Amp, Atm, Cfl, Ctx, Clo, Tri	-
ME5	baby bottle	Amc, Amp, Atm, Cfl, Ctx, Clo, Tri	-
BIR3	bottle teat	Tri	-

AMC, amoxicilin-clavulanic acid; AMP, ampicillin, ATM, aztreonam; CFL, cephalotin; CTX, cefotaxime; CLO, cloranfenicol; IPM, imipenem; TRI, trimethoprim. None of the isolates was resistant to amikacin, ceftazidime, ciprofloxacin, gentamicin, streptomycin, norfloxacin, tetracycline or tobramycin.

The presence of microbial biofilms has been associated with persistent infections, which responds poorly to the conventional antibiotic therapy [13]. Since the isolates in this study come from presumably sanitized baby bottles, the biofilm-forming ability was investigated by the Congo red method, as described previously by Freeman and colleagues [14]; based on this method, the slime producing bacteria appeared as black colonies, whereas non-slime producers remained non pigmented or reddish. Only the *Salmonella enterica* ATCC14028 strain, used as positive control, presented this characteristic. None of *Shigella* isolates showed biofilm production on this medium (Table 1). These results suggest that inadequate hygiene of utensils or even the ability of *Shigella* spp. to resist to chemicals used in the cleansing process within the lactary unit can constitute a significant factor in the persistence of these multidrug-resistant bacteria on utensils.

Preliminary investigations in the lactary showed some fails related to the correct sanitizing of utensils. After disinfection (washing with soap and immersion in boiled water), baby bottles and their accessories are placed reversed to dry and stay for a long time in this position until they are placed in closed containers. These utensils do not pass a final sterilization process (autoclaving). Other critical errors were also indicated, associated to water temperature for reconstitution and handling of left-over formula. Similar observations were described by Usai and coworkers [15] and suggest that these procedures compromise the safety of the formula, making the infant more susceptible to diarrheal diseases.

In conclusion, minimizing risks is essential to avoid possible foodborne diseases and the establishment of regular microbiological controls and proper sanitizing of utensils can facilitate obtaining the necessary sanitary quality. Attention to hygienic preparation of infant formula, hand washing, and boiling of baby bottles and bottle teats are interventions that can avoid or reduce contamination.

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