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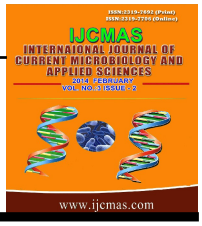

Original Research Article

Prevalence of nontyphoidal Salmonella serotypes and the antimicrobial resistance in pediatric patients in Najran Region, Saudi Arabia

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A B S T R A C T

Non-Typhoid Salmonella (NTS) commonly causes acute gastroenteritis, a major cause of morbidity, worldwide. This study aimed to determine the serotypes and the resistance patterns of NTS strains isolated from pediatric patients with acute gastroenteritis in Najran, Saudi Arabia. The study included 500 children aged < 5 years with diarrhea attending at Najran Maternity(NMCH).Stoolsamplesand Childr were collected from all patients and sent to microbiology department, College of Medicine, Najran University for detection of *NTS* serotypes and antimicrobial susceptibility. A total of 42 NTS isolates were identified. The highest isolation rate (40.5%)was in the age group ≤12 months. B (47.6%) and D (38.1%). In this study, 9.5% of *NTS* isolates were resistant to one antimicrobial agent and 14.3% were multidrug-resistant. The highest resistance of isolates was to tetracycline (71.4%), followed by ampicillin (54.8%) and chloramphenicol (26.2%). Salmonella Serotypes D and B predominated in pediatric NTS gastroenteritis. The study highlighted an increasing salmonella resistance to commonly used antibiotics. Continuous monitoring of serotypes resistance to antimicrobials is necessary for the public health implications of a potential spread of resistance clones.

Introduction

Diarrheal disease is an important health problem that causes high rates of morbidity and mortality in developing countries, and although mortality from infectious diarrhea has progressively been reduced, there has been a substantial increase in morbidity among young children worldwide (Kosek *et al*., 2003).

Among the bacterial causes of the diarrheal diseases, *Salmonella* continues to be a common causative agent. Non-typhoid salmonellosis (NTS) and typhoid fever are the most economically important food-borne diseases. While the incidence of typhoid fever is stable, cases of NTS are increasing worldwide (Olsen *et al*., 2001).

It is estimated that 93.8 million worldwide cases of acute gastroenteritis due to *Salmonella* species causing 155,000 deaths annually (Majowicz *et al*., 2010). Infection with NTS usually results in a self-limiting gastroenteritis in healthy children that does not require antibiotic therapy. However, serious sequelae, including systemic infections such as meningitis or septicemia were effective intravenous antibiotic therapy can be life-saving can occur and death may occur (Su *et al*., 2004; Hohmann, 2001).

In the last few years, there has been an alarming increase of *Salmonella* resistance to different antimicrobial agents and especially to those most commonly used, such as ampicillin (AMP), trimethoprim-sulfamethoxazole (SXT), chloramphenicol (CHL) or tetracycline; TET (Wannaprasat ., *et al*., 2011). Fluoroquinolones have been recommended for the treatment of Salmonella infections in adults where as the third generation cephalosporins are the drugs of choice for young patients

(Greko *et al*., 2009). The World Health Organization (WHO) has listed these drugs as critically important antibiotics for human health, highlighting the need for judicious use of these antibiotics in food animals (Collignon *et al*., 2009). The spread of resistant *Salmonella* serotypes is a relevant issue for pediatricians, because few therapeutic options are available for this age group (Majowicz *et al*. 2010; Su *et al*., 2004). There is very little information available from Saudi Arabia regarding the status of *Salmonella* in association with diarrheal diseases (Kambal, 1996; Fahad *et al*., 2001; Nasreldin Elhadi, *et al*., 2013). Identification of *Salmonella* serotypes and its pattern of antimicrobial susceptibility may provide epidemiological data for proper antimicrobial therapy (Majowicz *et al*., 2010).

This study aimed to determine the distribution and resistance patterns of NTS serotypes isolated from pediatric patients with acute gastroenteritis in Najran region, Saudi Arabia, during the period between January and July 2012.

Materials and Methods

This study included 500 children aged less than 5 years with diarrhea attending the outpatient clinic at Najran Maternity and Children's (NMCH). Diarrhea was defined as at least three loose stools in 24 hours, any number of watery stools, or 1 or 2 loose stools in 24 hours accompanied by at least one of the following symptoms: nausea, vomiting, abdominal cramps, or feve ºC. of Stool samples≥38 were collected from all patients and sent to the microbiology department at the College of Medicine, Najran University for detection of *Salmonella* enteropathogen serotypes. Stool samples were inoculated into Selenite-F enrichment broth (Difco, USA) for 4-6 hours at 37˚ and subcultured on MacConky and xylose lysine deoxycholate agar media (Difco) for Suspected 24 hours colonies on agaratmedia37˚Cwere biochemically identified as being *Salmonella* species by using the API 20E (Bio-Merieux, France) according to the manufacturer's instructions. *Salmonella* isolates were grouped with polyvalent antisera and serotyped based on somatic O and phases I and II flagellar antigens, by agglutination test with antisera (Wellcome Diagnostics, Dartford, UK) as specified by the White-Kauffman-Le Minor scheme.

All *Salmonella* serotypes were subjected to antimicrobial susceptibility testing by disc diffusion method as recommended by

Clinical Laboratory Standards Institute (CLSI) on Muller-Hinton agar (Oxoid, France) with commercial antibiotic discs (Oxoid). The antibiotic discs used in this study were ampicillin; AMP (10 µg), amoxicillin/clavulanic acid (Augmentin); AMC (20/10 mg), ceftriaxone; CRO (30 µg), chloramphenicol; CHL (30 µg), tetracycline; TET (30 µg), nalidixic acid; TET (30 µg), Gentamycin; GEN (10 µg), trimethoprim-sulfamethoxazole; SXT (30 µg) and ciprofloxacin; CIP (5 µg). Multidrug-resistance was defined as resistance to three or more antimicrobials subclasses.

Results and Discussion

During the study period, a total of 42 NTS isolates were identified. The highest isolation rate (17 isolates; 40. followed by 9 isolates (21.4%) in children aged 13-24 months, 6 isolates (14.3%) in each of 37-48 and 49-60 months age groups and 4 isolates (9.5%) in children aged 25-36 months. The most prevalent NTS serotype was B isolated from (20 isolates, 47.6%), followed by serotype D (16 isolates; 38.1%) and serotype C (4 isolates; 9.5%), table (1).

The antimicrobial resistance of all NTS serotypes is presented in table (2). Overall, 9.5% (4/42) of NTS isolates were resistant to one class of antibiotics and 14.3% (6/42) were multidrug-resistant, defined as resistance to three or more antibiotic classes. The highest resistance of all isolates was to tetracycline (71.4%), followed by ampicillin (54.8%), chloramphenicol (26.2%) and gentamicin (19%).

Despite the development in sanitation and hygiene, NTS illnesses continue to impose a significant burden both the developed on and the popul developing countries (Majowicz SE, *et al*. 2010). In this study, > 85% of NTS isolates were serogroup B and D. This distribution is similar to that shown in previous reports from the Kingdom (diseases (Kambal, 1996; Fahad *et al*., 2001; Nasreldin Elhadi, *et al*., 2013). According to Salm-Surv (the WHO supported food borne disease surveillance network) data from 2001 to 2005, *S. rnteritidis* (serogroup D) was the most common serotype worldwide (65% of the isolates), followed by *S. typhimurium*; serogroup B (12%) and S. Newport (4%) (Nasreldin Elhadi, *et al*., 2013). In this WHO report, *S. enteritidis* and *S.typhimurium* represented 26% and 25% of the isolates in Africa, respectively. In Asia, Europe and Latin America, *S. Entiritidis* was the most frequent isolate (38%, 87% and 31%, respectively). In North America *S. typhimurium* was the most frequently reported (29%) followed by *S. enteritidis* (21%) and other *Salmonella* spp. (21%).

In our study, which is the first report from this region of the kingdom of Saudi Arabia, the majority (62%) of NTS isolates were from children less than 2 years of age. This is in agreement with other reports (Huang *et al*., 2012; Jabeen *et al*., 2010; Vithayasai *et al*., 2011). Previous studies reported that NTS patients <2 years of age demonstrated severe clinical manifestations, including a higher incidence of bloody stool, mixed infections, and extra-intestinal manifestations and higher rates of complications, were on this study, none of the patients developed systemic diseases. Early childhood is characterized by an increased susceptibility to infectious diseases, and this has been attributed to both the immaturity of the immune system at birth and to the sluggish development of

immunocompetence during the postnatal and early childhood years. This vulnerability to infections appears to be particularly pronounced in relation to intracellular pathogens, reflecting the functional immaturity of cell-mediated immunity (Upham *et al*., 2002). Furthermore, gastric hypoacidity, the home environment and intra-familiar transmission also play a role in the development of NTS in younger children (Hohmann, 2001).

Antimicrobial resistance in NTS serogroup is a global problem. In the present study, 9.5% of *NTS* isolates were resistant to one class of antimicrobials and 14.3% were multidrug-resistant. Similar to previous reports (Majowicz *et al*. 2010; Su *et al*., 2004; Huang *et al*., 2012; Jabeen *et al*., 2010; Vithayasai *et al*., 2011), *S. typhimurium* was the most frequent resistant serotype. Regardless of *NTS* serotype, the resistance rates towards therapeutically relevant first-line antibiotics (AMP; 54.8% and CHL; 26.2%), although relatively higher than reported in previous Saudi studies (AMP; 16%-46.6% and CHL; 11%-17.2%) (Kambal, 1996; Fahad *et al*., 2001; Nasreldin Elhadi, *et al*., 2013), still lower compared to reports from other Asian countries like Malaysia, Thailand and Vietnam (AMP, 23–100%, CHL; 36%-100%) (Van *et al*., 2012). Interestingly, only 4.8% of the *NTS* isolates in our study were resistant to SXT, compared to much higher rates noted in other Saudi studies (11%-38%) (Kambal, 1996; Fahad *et al*., 2001; Nasreldin Elhadi, *et al*., 2013) and other asian countries like Thailand and Vietnam (Van *et al*., 2012). Ciprofloxacin (CIP) and third-generation cephalosporins are the antimicrobial agents recommended for the treatment of complicated extra-intestinal infections. The rate of resistance to ceftriaxone (CRO) and CIP found in our study is higher than that reported in previous Saudi studies (Kambal, 1996; Fahad *et al*., 2001;). Our finding is of great concern, as continued emergence and dissemination of *NTS* strains with reduced susceptibility to these precious antibiotics are likely to have worrying clinical consequences (Parry *et al*., 2008).

In conclusion, NTS Serotypes D, B and C predominate as causative agents of pediatric salmonella gastroenteritis in our region. The majority of the isolates were from children less than 2 years of age. This study highlighted an increasing NTS resistance to commonly available antibiotics which are of public health concern and the pediatricians should be aware of the local epidemiological data of NTS and carefully review the results of antimicrobial susceptibility testing once available. have important implications on therapeutic options. Although the majority of isolates are still susceptible to ceftriaxone and ciprofloxacin, continuous monitoring of serotype resistance is of paramount importance for the public health implications of a potential spread of resistant clones.

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