Investigating Antimicrobial Resistance in Salmonella Meningitis: A Nigerien Perspective

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Abstract

Salmonella meningitis prognosis is poor and the choice of adequate antibiotic therapy is difficult in developing countries where laboratory testing is not accessible. This study aimed to evaluate, the antibiotic susceptibility pattern of *salmonella* isolated from CSF in meningitis suspected cases and to identify the best drug option. From 2011 to 2015, 6630 CSF collected were sent at CERMES, during laboratory-based surveillance. All turbid and freshly collected CSF from under 5 years patients were subjected to standard bacteriological method for isolation and characterization of meningitis etiologies. A total of 11/6630 *Salmonella* strains were isolated during this study. This accounted for 0. 2% of the overall CSF analyzed during the 5 years of study. Most of the patients (72.7%) were less than 2 years old. The average age of children was 2.63 years with an extreme of 1 month to 14 years and the sex ratio M/F was 0.83. The antimicrobial susceptibility was performed in vitro, to all the 11 *salmonella* isolated. The result revealed that 9/11(81.8%) strains were susceptible to ceftriaxone, Amikacin, and Cefoxitin. All the isolates (100%) were sensitive to Imipenem, Nalidixic acid, and Ciprofloxacin. Two strains of *salmonella* (18.2%) belonged to extended-spectrum beta lactamase (ESBL) producing group.The maximum resistance was observed against Ampicillin (100%), Amoxicillin/clavulanic acid (72.7%) and Gentamicin (27.3%).The Ampicillin MIC value showed 100% resistance to all the strains tested. This study confirmed that ceftriaxone may be used to treat *Salmonella* meningitis.The isolation of ESBL *salmonella* strain may be an alarm indicating the worldwide spray of multi-resistant bacterium. However, the limitation in the use of cephalosporin may prevent this increasing resistance.

Introduction

Salmonella is a widespread food born disease encountered frequently in developing country with sanitation problem. Meningitis due to *salmonella* is relatively uncommon but it is of importance because of high mortality rate (Though the disease is known to be the fourth cause of meningitis in children, after *Neisseria meningitidis* (Nm), *Streptococcus pneumoniae (S.pn)*, and *Haemophilus influenzae type b*

Christensen & Frederiksen 1988). (*H.i b*) (OwusuOfori & Scheld 2003). Salmonellosis is a public health problem highly associated with septicemia in children and adult (Mahalakshmi 2013; Kanchanapongkul 1995) The infection may spread from intestine to blood stream causing bacteremia (Altun *et al*. 2014). Pregnant woman may also transmitted the infection to his baby as carrier (Olivares Lopez *et al*. 1981). The disease prognosis is poor and the choice of adequate antibiotic therapy is difficult where laboratory testing is not accessible (Fuller *et al*. 2003; Khemiri *et al*. 1984). Antimicrobial agents previously used seem to be not efficient. Though, there is limited data from Africa (LONGE *et al*. 1984). *Salmonella* antimicrobial resistance has been reported from both developed and particularly in developing countries where the choice of antimicrobial treatment may be a problem (Kanchanapongkul 1995). In fact, some isolates were now resistant to

ampicillin and not all were sensitive to Gentamicin and Chloramphenicol (Fuller *et al*. 2003). This antimicrobial resistance has been a serious public health problem (S. & G. 1951; Mahalakshmi 2013). New treatment with ceftriaxone and other third generation cephalosporin were recommended (Anon 2015). The combination of quinolone and ceftriaxone were suggested as an alternative solution (Price *et al*. 2000; OwusuOfori & Scheld 2003). *Salmonella* meningitis is rare or often neglected in Niger. It's then difficult to estimate the fatality rate due to *salmonella* meningitis. However, the disease mostly occurs in neonates and young infants (Ali *et al*. 2017).

So far, no recommended treatment against *Salmonella* meningitis was available in Niger. The ministry of health was recommended the use of Ceftriaxone 2g daily for a minimum of five days in the treatment of meningococcal meningitis (OMS, 2015). Thus Patients may be treated with common used antibiotics against meningococcal meningitidis.

Objectives:

This work aimed to evaluate in vitro, the antibiotic susceptibility pattern of *salmonella* isolated from CSF in meningitis suspected cases and to identify the best drug option.

Material and methods

Samples collection

This retrospective study was conducted at Center for medical and Health research (CERMES), the National Reference Laboratory for meningitis in Niger from January 2011 to December 2015. Cerebrospinal spinal fluids (CSF) were collected nationwide from suspected cases and sent to CERMES for analysis. All freshly collected CSF were subject to culture by standard bacteriological method for isolation and characterization of bacterial meningitis etiologies (culture, Gram, latex, antimicrobial sensibility). Socio-demographic information and history of the infection were also collected and analyzed in the study.

Salmonella isolation and identification

CSF were cultured on Blood Agar Plate which is a trypticase soy agar (TSA) plate containing 5% sheep blood; and on chocolate agar plate supplemented with hemin (X factor) and nicotinamide-adenine-dinucleotide (NAD; V factor). Plates were then incubated in candle jar for 18-24 hours at 35-37°C with 5% CO_2. All media were previously tested for growth and sterility with reference strains as part of quality control. All Bacteria that appeared Gram Negative rod shaped bacilli after Gram staining, were considered as suspect of *salmonella* and purified on nutrient agar after 18 to 24 hours period of incubation at 37°C.

Suspected *salmonella* colonies were confirmed by biochemical reactions (motility, indole production, lysine decarboxylase, carbohydrate fermentation) using 20E API kit (Biomérieux, Marcy l'étoile, France) and serotyped by slide agglutination with *Salmonella* antigen antisera (Williamson & Murti 1990; GEORGES *et al*. 1AD; Berk & McCabe 1980). Then, agglutination test was carried out using a panel of *salmonella* antisera according to Kauffmann and White scheme.

Antibacterial susceptibility

Antimicrobial susceptibility test was performed by Kirby Bauer disk diffusion methods in respect to European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2016). Identified pure culture of Salmonella was inoculated on Muller-Hinton agar plate (Liofilchem, Italy) with a depth of 4mm.Bio-Rad antibiotics discs were used to screen for susceptibility according to EUCAST 2016. *Escherichia coli* American Type Culture Collection (ATCC25922) were used as quality control strains and for Minimal Inhibitory Concentration (MIC) determination.

All *Salmonella* isolates were tested for resistance to 9 antimicrobial agents: Ampicillin (AM,10 µg), Amikacin (AKN,30µg), Amoxicillin/clavulanic acid (AMC,10 µg), Ciprofloxacin (CIP,5 µg), ceftriaxone (CRO,30µg), Cefoxitin (CTX,30µg) Gentamicin

(GEN,10µg), Imipenem (IPM,10µg), and Nalidixic acid (AN,30µg), according to recommendations of Clinical and Laboratory Standards Institute (Anon n.d.).

In case of resistance the MIC was performed by using E-test (Bio-Mérieux). The inhibition zones were measured by Venire Caliper to categorize the isolate as susceptible, intermediate, resistant or non-susceptible (Baud & Aujard 2013; Watson 1957). All the isolates were conserved in Trypticase Soy Broth + 15% glycerol at -80°C for further research work.

Results

Salmonella meningitis is uncommon in Niger. A total of 11/6630 *Salmonella* strains were isolated during 2011-2016 meningitis based surveillance. The disease is rare and accounted for 0. 2% of the overall CSF analyzed at CERMES during the 5 years of study. Most of the patients were children (72.7%) less than 2 years old.

The average age of children was 2.63 years with an extreme of 1 month to 14 years and the sex ratio M/F was 0.83. of the eleven *salmonella* species detected, we found 7 (63.7%) *Salmonella typhi*, 2 (16.7%) *Salmonella* spp, 1(8.3%) *Salmonella paratyphi* A and 1(8.3%) *Salmonella paratyphi* B. The antimicrobial susceptibility was performed to all the 11 *salmonella* isolated and the results were shown in Fig. 1. In current study, the antimicrobial susceptibility testing in vitro, revealed that 9/11 (81.8%) strains were susceptible to ceftriaxone, ciprofloxacin and Cefoxitin. All the isolates (100%) were sensitive to Imipenem and Nalidixic acid.

Two strains of *salmonella* (18.2%) belonged to Extended-Spectrum Beta Lactamase (ESBL) producing group as shown in Fig. 1. The maximum resistance was observed against Ampicillin (100%), followed by Amoxicillin/clavulanic acid (72.7%), Amikacin (27.3%) and Gentamicin (27.3%). The Ampicillin MIC value (0.0µg/ml) also shown 100% resistant to all the 11 strains tested (Fig. 2).

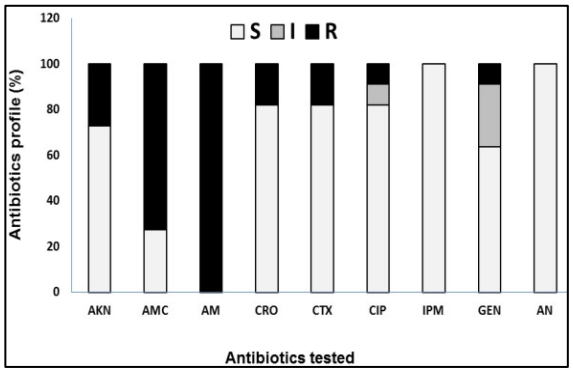

Fig. 1. Antibiotics resistance profile of *Salmonella* isolates collected from CSF.

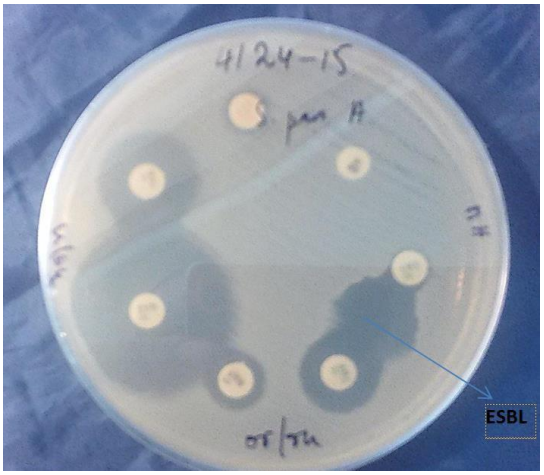

Fig. 2. Broaded spectrum Beta lactamase *Salmonella paratyphi* (ESBL).

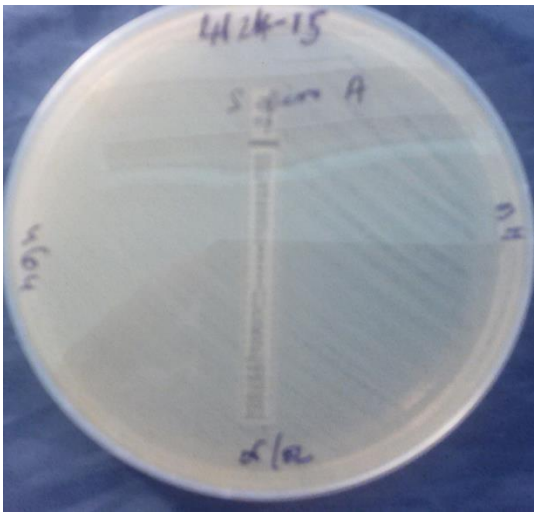

Fig. 3. Ampicillin Minimum Inhibition Concentration (MIC).

Discussion

The treatment of *salmonella* meningitis is presently difficult since the disease is often neglected (Workman *et al*. 1999; Price *et al*. 2000). *Salmonella* meningitis is poorly or not reported from Niger. The bacterium may be neglected or not notified as cause of meningitis due to the absence of modern laboratory facilities in many areas (ARSLAN Ahmed, 2012). Another reason is that, in case of any symptom such as headache, fever, relative to meningitis, medical and health professionals evoke *Neisseria*, *streptococcus* and *Heamophilus* bacteria. The disease occurs particularly in the dry season from December to June.

Young children were mostly affected in endemic area as revealed by other studies worldwide (Doctor *et al*. 2001; LOW *et al*. 1984). The reason may be due to mother carrier, hygiene and alimentation problem A. Ali *et al* (2017). Salmonellosis infection is almost always by fecooral route (Kumar *et al*. 2014).

Most serogroupes isolated in this study, were known to cause typhoid fever (typhi and paratyphi). This may probably be explained by sanitation problem. In this study all the *salmonella* isolated were sensitive to Imipenem and ciprofloxacin used to treat salmonellosis in this part of the world. This finding is in concordance with the result of Sangaré *et al*, (2007) in Burkina Faso, and Olafemi *et al*, (2000) in Nigeria, but different from Helms *et al*, (2002) result in Ghana. About 9/11 strains (81.8%) were sensitive to ceftriaxone the most used and recommended antibiotic for meningitis treatment in Niger (WHO, 2015). This antibiotic with good diffusion may be efficient against *salmonella* (Owusu-Ofori & Scheld 2003; Fuller *et al*. 2003). This work showed that early treatment with ceftriaxone together or not with Ciprofloxacin could lead to good cure rate (Thiombiano *et al*. 2007; Coldiron *et al*. 2017). However, the previously used antibiotics Ampicillin, and Gentamicin were inefficient (Owusu-Ofori & Scheld 2003). Ampicillin has been primarily used as drug of choice in the treatment of meningitis in Niger, though some rural health centers still used it in case of emergency. According to our result, all *salmonella* strains tested were resistant to Ampicillin (100%) and (27.3%) were resistant to Gentamicin as stated early by Elisabeth Price (2000) and Scheld (2003) in Ghana. The result of MIC showed that Ampicillin was resistant and may be inefficient in the treatment of *Salmonella* meningitis in this part of the meningitis belt as told by Owusu-Ofori and Scheld 2003 from Ghana. In our study, 2(0.2%) *salmonella* isolates were found to produce an extended-spectrum beta lactamase (ESBL).

This may probably indicate the emergence of new multi-resistant *salmonella* strain to third generation cephalosporin such as ceftriaxone and Amoxicillin/clavulanic acid. According to our finding, these ESBL producers were 100% sensitive to carbapenems. Similar data about Imipenem were presented by Alipourfard *et al* (2010) in Bangladesh.

Conclusion

This study conducted for the first time in Niger revealed and confirmed that ceftriaxone may be suggested in the treatment of *Salmonella* meningitis either alone or in combination with Ciprofloxacin. This study also showed that quinolones were very efficient whereas ampicillin was completely inefficient in the treatment of *Salmonella* meningitis. Thus, the isolation of ESBL *salmonella* strain may be an alarm indicating the worldwide spray of multi-resistant bacterium. However, the limitation in the use of cephalosporin may prevent this increasing resistance.

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