

## Full Length Research Paper

# Prevalence of beta-lactamases in *Salmonella* and *Shigella* species in different hospitals in Anyigba, Kogi State, Nigeria

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Received 16 October, 2014; Accepted 12 January, 2015

**Beta-lactams are the most widely used antibiotics. Resistance to beta-lactam antibiotics is an increasing problem worldwide and beta-lactamase production is the most common mechanism of resistance to these drugs. Species of *Salmonella* and *Shigella* isolated from hospital patients in Anyigba, Kogi State, were analyzed for the presence of beta-lactamases using Nitrocefin, Iodometric, Acidimetric and Double Disc Synergy methods. Results showed that 9 (81.82%) of *Shigella* species and 22 (61.11%) of *Salmonella* species were beta-lactamase producers with 3 (27.27%) of *Shigella* species and 20 (55.56%) of *Salmonella* species producing extended spectrum beta-lactamases (ESBLs). Beta-lactamase production is an acknowledged threat to modern medicine especially antibiotic usage.**

**Key words:** *Salmonella*, *Shigella*, beta-lactams, beta-lactamase, extended spectrum beta-lactamases.

## INTRODUCTION

In Gram-negative pathogens,  $\beta$ -lactamases remain the most important contributing factor to  $\beta$ -lactam resistance, and their increasing prevalence, as well as their alarming evolution seems to be directly linked to the clinical use of novel sub-classes of  $\beta$ -lactams (Medeiros, 1997).

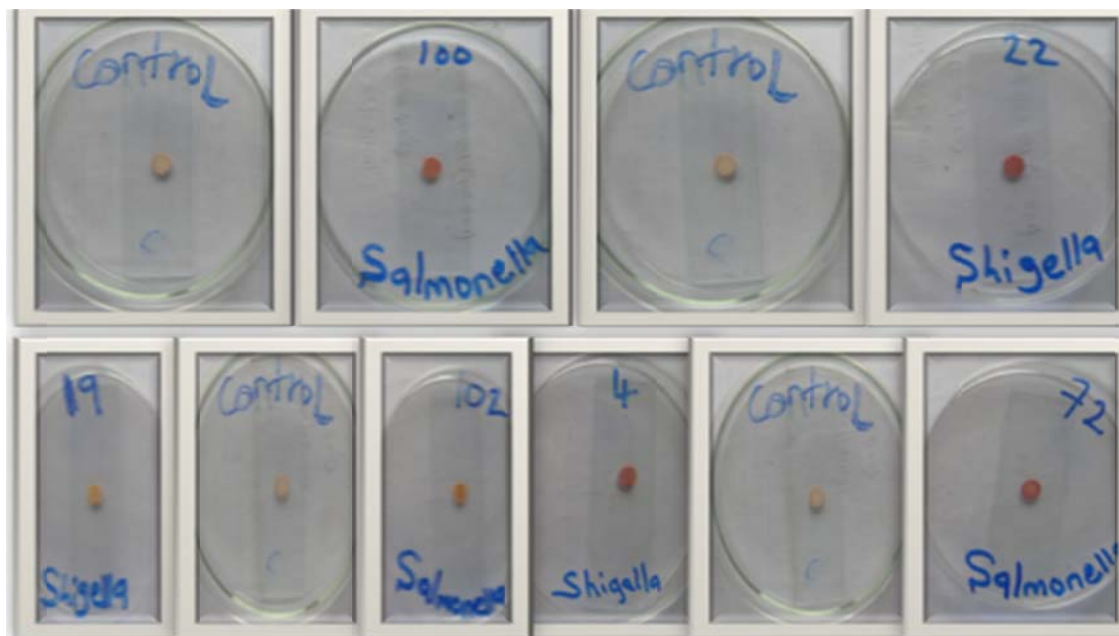
$\beta$ -Lactamases are bacterial enzymes that inactivate  $\beta$ -lactam antibiotics by hydrolysis, which result in ineffective compounds (Bush, 2001). Beta-lactam antimicrobial agents such as Penicillins, Cephalosporins, Monobactams and Carbapenems, are among the most common drugs for the treatment of bacterial infections and account for over 50% of global antibiotic consumption (Kotra et al., 2007).

Bacterial resistance to  $\beta$ -lactam antibiotics has significantly increased in recent years and has been

attributed to the spread of plasmid mediated  $\beta$ -lactamases. Some of these organisms have produced new forms of the older enzymes such as the extended-spectrum  $\beta$ -lactamases (ESBLs) that can hydrolyze newer Cephalosporins and Aztreonam (Paterson and Bromo, 2005).

ESBLs are enzymes that mediate resistance to extended spectrum (third generation) Cephalosporins such as Ceftazidime, Cefotaxime and Ceftriaxone as well as Monobactams such as Aztreonam. These ESBLs have been found worldwide in many different genera of enterobacteriaceae (Bradford, 2001). More than 200 different natural ESBLs variants are known in an increasing variety of Gram-negative species (Bradford,

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**Figure 1.** Nitrocefin disc (Remel Europe, Ltd UK) results for the detection of beta-lactamase in *Salmonella* and *Shigella* species.

2001) with their distribution being far from uniform (Marchandin et al., 1999).

Reports have shown that the resistance of gastroenteric *Salmonella* and *Shigella* strains to antimicrobial agents is in large part due to the production of ESBLs encoded on plasmids, as well as on the chromosome (David and Frank, 2000; Rodriguez et al., 2004).

With  $\beta$ -lactams being the most frequently prescribed antimicrobials, the emergence of ESBLs producing organisms in clinical infections can result in treatment failure constituting a serious threat to current  $\beta$ -lactam therapy (Yusha et al., 2010) especially in areas such as Anyigba where treatment options are not many because of dearth of resources. This study was therefore carried out to determine the occurrence of beta lactamases among species of *Salmonella* and *Shigella* in hospitals in Anyigba where these organisms are assuming endemic status.

## MATERIALS AND METHODS

### Sample collection and isolation of organism

Total of two hundred and fifty (250) faecal samples were collected aseptically from patients attending different hospitals in Anyigba, Kogi State, Nigeria.

### Isolation and Identification of the organisms

Samples were inoculated onto *Salmonella-Shigella* agar plates, MacConkey agar plates and incubated at 37°C for 24 h.

All isolates were Gram-stained, examined microscopically and subjected to biochemical and other tests including the triple sugar iron agar test (TSI), catalase activities, indole production, carbohydrate fermentation, motility test and urease production for the identification of the test organisms (Cheesbrough, 2004). Identity of isolates was further verified using CHROM agar™.

### Beta-lactamase detection tests

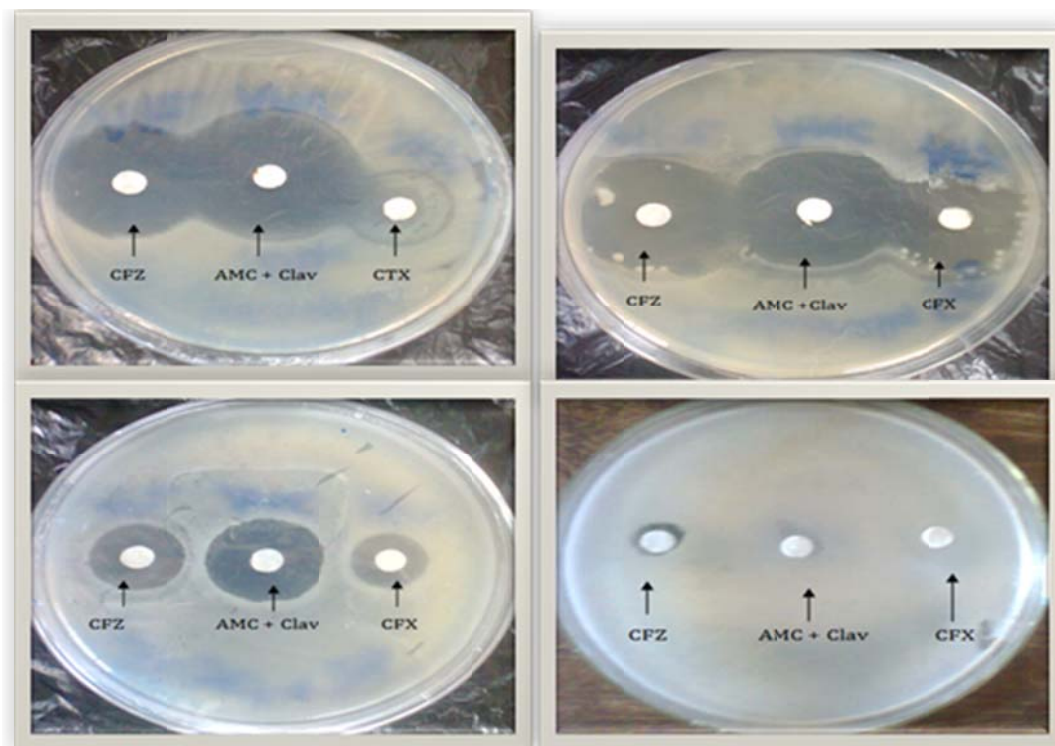
Based on the antibiogram of isolates (data presented in another report), 36 isolates of *Salmonella* and 11 isolates of *Shigella* were subjected to the following tests: All tests were done in triplicate and with controls.

### Nitrocefin test procedure

The  $\beta$ -lactamases were visualized by using Nitrocefin Disk (Remel Europe, Ltd UK) (Figure 1). The discs were moistened with a loopful of deionized water.  $\beta$ -Lactamases production was inferred when the disk turned pink/red. A sterilized forceps was used to place the disk on clean glass slide in an empty Petri dish. The colonies of the test bacteria were picked from overnight Mueller Hinton agar plates and smeared on the disk and incubated for 1-2 min at room temperature (25°C). Positive result was inferred when the disk turned red from yellow showing hydrolysis (Nitrocefin is a Chromogenic Cephalosporin that changes colour from yellow to red with hydrolysis) (Cheesbrough, 2004; Livermore and Brown, 2001).

### Iodometric test using paper strip method

About 0.2 g of soluble starch was added to 100 ml of distilled water and dissolved by boiling. After cooling, 1 g of benzylpenicillin was added. Filter papers (1×5 cm, Whatman No. 3) were soaked in this solution, then air dried for 2 h and stored at 4°C. The strips were



**Figure 2.** Detection of ESBL production using double disc diffusion synergy tests. Disc: left, Ceftazidime, 30 µg; centre, Amoxicillin + clavulanate, 20 + 10 µg; and right, Cefotaxime, 30 µg.

**Table 1.** The occurrence of beta-lactamase production in *Salmonella* and *Shigella* species.

| Bacterial species         | No. tested | No. of $\beta$ -Lactamase Producers | Percentage (%) occurrence | No. of non $\beta$ -Lactamase Producers | Percentage (%) of non-occurrence |
|---------------------------|------------|-------------------------------------|---------------------------|---|----------------------------------|
| <i>Salmonella</i> species | 36         | 22                                  | 61.11                     | 14                                      | 38.89                            |
| <i>Shigella</i> species   | 11         | 9                                   | 81.82                     | 2                                       | 18.18                            |

moistened with 2% (w/v) iodine in 53% (w/v) aqueous potassium iodide before use. They were then smeared with colonies (colonies of *Salmonella* and *Shigella* species) from an overnight culture plates over an area of 2 – 3 mm. Decolourization within 5 min indicates  $\beta$ -lactamase activity (Livermore and Brown, 2001).

#### Acidimetric test using paper strip method

Filter paper (Whatman No.1) was cut into 5×1 cm strips and soaked in a freshly prepared solution containing equivalent of 125 g/L benzylpenicillin, 0.1% (w/v) bromocresol purple and 1.25 mM NaOH. The strips were dried and stored at 4°C. The strips were moistened with distilled water, *Salmonella* and *Shigella* species from agar (not broth) cultures were smeared on the strips and development of yellow colour within 5 min indicated  $\beta$ -lactamase activity (Livermore and Brown, 2001).

#### Detection of extended spectrum $\beta$ -lactamases

The presence of ESBL was detected by the Double Disc Synergy Test (DDST) (Figure 2). Plates were inoculated with test isolates, as

described for routine susceptibility testing. Discs containing co-amoxiclav 20 + 10 µg and Ceftazidime 30 µg were placed at a distance of 27 mm apart. A disc containing a different Cephalosporin (Cefotaxime) was placed on the opposite side of the co-amoxiclav disc. The plates were incubated overnight at 37°C, and ESBLs were inferred when the cephalosporin zones were expanded by the clavulanate (Livermore and Brown, 2001).

#### Statistical analysis

All the data were subjected to one way analysis of variance (ANOVA) to determine significance or otherwise of the occurrence of ESBLs harbouring isolates vis-a-vis the total number tested.

## RESULTS

Table 1 shows that 22 (61.11%) *Salmonella* species are  $\beta$ -lactamase producers, 14 (38.89%) were non  $\beta$ -lactamase producers. For *Shigella* species, 9 out of 11 isolates (81.82%) were producers of  $\beta$ -lactamase while

**Table 2.** Comparison of three tests for beta-lactamase detection.

| Organisms                    | Iodometric test |         |         | Nitrocefin test |         |         | Acidimetric test |         |         |
|------------------------------|-----------------|---------|---------|-----------------|---------|---------|------------------|---------|---------|
|                              | No. tested      | No. pos | No. neg | No. tested      | No. pos | No. neg | No. tested       | No. pos | No. neg |
| <b><i>Salmonella</i> sp.</b> |                 |         |         |                 |         |         |                  |         |         |
| MDR                          | 16              | 13      | 3       | 16              | 13      | 3       | 16               | 13      | 3       |
| I                            | 9               | 4       | 5       | 9               | 5       | 4       | 9                | 5       | 4       |
| MDS                          | 11              | 4       | 7       | 11              | 4       | 7       | 11               | 4       | 7       |
| <b><i>Shigella</i> sp</b>    |                 |         |         |                 |         |         |                  |         |         |
| MDR                          | 4               | 4       | 0       | 4               | 3       | 1       | 4                | 4       | 0       |
| I                            | 2               | 0       | 2       | 2               | 1       | 1       | 2                | 1       | 1       |
| MDR                          | 4               | 3       | 1       | 4               | 3       | 1       | 4                | 3       | 1       |

MDR, Multi drug resistance; I, intermediate category; MDS, multi drug susceptible; No, number; Pos, positive; Neg, negative.

**Table 3.** Percentage occurrence of ESBLs producers among the isolates via double disc diffusion synergy test.

| Isolates                  | Number screened | Number positive | Occurrence of ESBLs present (%) |
|---------------------------|-----------------|-----------------|---------------------------------|
| <i>Salmonella</i> species | 36              | 20              | 55.56                           |
| <i>Shigella</i> species   | 11              | 3               | 27.27                           |
| Total                     | 47              | 23              | 82.83                           |

only 2 isolates (18.18%) were non  $\beta$ -lactamase producers.

Table 2 shows the results of three different analytical methods of  $\beta$ -lactamase production: colorimetric method (43 isolates), the acidimetric method (43 isolates) and the iodometric method (43 isolates). Colorimetric (Nitrocefin) method test gave positive result within one second to two minutes, the acidimetric method gave positive result within 1 - 5 min; and the iodometric gave positive result within 1 - 5 min. All the methods showed similar results except for a few differences among *Shigella* and *Salmonella* species.

The ESBL was detected by the double disc diffusion synergy test (DDST) as shown in Table 3. The result indicated that out of 36 *Salmonella* species, 20 (55.56%) were ESBL positive and out of 11 *Shigella* species 3 (27.27%) were ESBL producers.

Expanded cephalosporin zones of inhibition by clavulanate shows presence of ESBL.

## DISCUSSION

Intestinal colonization by organisms capable of producing beta-lactamases is increasingly becoming a worrying problem (Gyansa-Lutterodt, 2013). Our study has demonstrated the presence of these enzymes in two bacterial taxa that are assuming endemic clinical status in the study area. A similar activity has been reported among *Salmonella* species in other countries like Turkey, Nepal and South Africa (Irajian et al., 2009). In a related

study in Ghana, Obeng-Nkrumah et al. (2013) found that more than 87% of *Salmonella* strains studied were ESBL producers.

The challenge is that these enzymes have been reported to bring about resistance to Cefotaxime, Ceftazidime and Aztreonam and are encoded on conjugative plasmid, transposons or integrons- genetic materials which can spread readily (Irajian et al., 2009).

Previous studies have shown that resistance to broad spectrum  $\beta$ -lactams is highly mediated by ESBLs enzymes, increasing the world health problem in clinical settings (Valverde et al., 2008). In the present study, 55.56% of *Salmonella* and 27.27% of *Shigella* were ESBL producers. This is in relation to previous reports showing that, although prevalence and phenotypic characteristics among clinical isolates may vary between geographical areas, ESBLs are widely spread (Bradford, 2001). In the last two decades, they have emerged as major contributors of drug resistance. As compared to their prevalence among *E. coli* and *Klebsiella* spp., they have also emerged in *Salmonella* as important clinical problems (Uma et al., 2010).

The high ESBL production demonstrated in this report therefore showed that *Salmonella* and *Shigella* species are competing with *Klebsiella* species as ESBL producers and care should be taken when handling such isolates. According to earlier reports by Spanu et al. (2002) and Zaki (2007), many ESBL producers carry other genes that confer resistance to other antimicrobial agents such as aminoglycosides and fluoroquinolones. In a separate

report, our findings showed that drug resistance was higher among beta-lactamase producing strains of *Salmonella* and *Shigella* than non-producers.

Drug resistance is of increasing concern in modern medicine. Beta-lactams are the most widely used antibiotics and beta-lactamases are the greatest source of resistance to them. Beta-lactamase activities are widely spread among *Salmonella* and *Shigella* species and have been strongly supported by the results of this work. This has obvious public health implications especially in resource-poor areas such as Anyigba and its environs where this research was focused.

### Conflict of interests

The authors did not declare any conflict of interest.

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