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(RESEARCH ARTICLE)



Extracellular enzymes and β -lactamase screening of bacterial isolates cultured from urine samples of immunocompromised women

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Abstract

Enzymes intricate by organisms are used to inactivate and destroy antibiotics. This study determined some virulence factors elaborated by the bacterial isolates cultured from urine samples of HIV seropositive pregnant women that attended antenatal clinic of the Ondo State Specialist Hospital, Akure. Screening for extracellular enzymes and extended spectrum beta lactamase (ESBL) of the isolate was carried out using standard method and disk approximation method respectively. The extracellular enzymes such as lipase, protease and DNase were detected in the three bacterial genera used. ESBL was found in *S. aureus* and *E. coli*. The study concluded that most of the bacterial isolates cultured from urine samples of HIV seropositive pregnant women in the study area were found to have these virulence factors.

Keywords: Screening; Extracellular enzymes; Bacteria; HIV seropositive

1. Introduction

Enzymes intricate by organisms are used to inactivate and destroy antibiotics. Beta-lactamases are enzymes capable of hydrolyzing beta-lactam antimicrobials and at the same time inactivating them [1]. There are hundreds of different beta-lactamase enzymes which differ in terms of the specific beta-lactam antimicrobials they are able to inactivate [1]. Extended-spectrum beta-lactamases (ESBLs) are beta-lactamase enzymes capable of hydrolyzing extended-spectrum or third generation cephalosporins (e.g., ceftriaxone and/or ceftazidime) [2]. They do not hydrolyze carbapenems and they are susceptible, in turn, to inhibition by conventional beta-lactamase inhibitors (clavulanate) [3].

ESBL producing organisms remain an important cause of therapy failure with beta-lactam antibiotics and have a serious impact on infection control [4]. Therefore, the detection of ESBL producing organisms and the correct choice of antibiotics is important [5]. Large numbers of outbreaks due to ESBL producing organisms have been reported around the world and their prevalence is increasing [6]. The extended spectrum beta-lactamases (ESBLs) enable certain Gram-negative bacteria to inactivate cephalosporins as well as broad-spectrum penicillins and monobactams (aztreonam). ESBL production by Gram-negative organisms is an emergent, worldwide problem [7] and the presence of these enzymes has an impact on the efficacy of β -lactam therapy [2, 8]. ESBLs can present variations in the *in vitro* pattern of resistance to β -lactam agents conferred to the samples that encode them [9].

Recovery of antibiotic-resistant, especially to β -lactams, strains of Enterobacter is reported to be on the increase [10], particularly during therapy with β -lactam agent [2]. The molecular basis for the resistance in these Enterobacter isolates is a mutation to an ampD gene that normally prevents high-level expression of the chromosomal β -lactamase encoded by an ampC gene [5]. Studies done by Olagoke *et al.* [11] stated that plasmid induces the production of extra-cellular

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enzymes called β -lactamases, and also encoded resistance genes which are often located within genetic elements. The aim of the study is to screen and determine some virulence factors elaborated by the bacterial isolates.

2. Material and methods

2.1. Materials

Bacterial isolates (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas* spp) cultured from the urine samples of HIV seropositive pregnant women attending antenatal clinic at the Ondo State Specialist Hospital, Akure, South western, Nigeria were used for the study. Each bacterial isolate was verified using cultural morphology, Gram's staining, selective media and differential media as well as biochemical tests.

2.2. Determination of extracellular enzyme production

Determination of extracellular enzyme production of all bacterial isolates was carried out for their ability to produce protease, lipase and DNase using specific media (skimmed milk agar, tributyrin agar and DNase agar respectively).

2.2.1. Screening for lipase production

All the bacterial isolates were streaked on solid tributyrin agar medium [12] containing (g/L), peptone from meat 2.5; peptone from casein 2.5; yeast extract 3.0; agar-agar 15.0 and 10.0 ml tributyrin (glycerol tributyrate), pH was adjusted to 7.5. The presence of clear zone around the colonies indicated lipase production. The diameter of clear zone was measured for each colony.

2.2.2. Screening for protease production

All the bacterial isolates were streaked on solid skimmed milk agar medium containing peptic digest of animal tissue 5.0, beef extract 1.5, yeast extract 1.5, sodium chloride 5.0, agar 15.0 and skimmed milk 10.0 in g/l.

2.2.3. Screening for DNase

The 1 to 2 colonies of 24 h old culture of each bacterial isolate was inoculated on set DNase plates by streaking. The plates were incubated at 37 °C for 24 hour. After 24 h after which it was flooded with 1 N HCl. A zone of inhibition indicates DNase activity.

2.3. Screening for extended spectrum betalactamase

Screening for extended spectrum betalactamase was done by using double disk approximation method [13]. Test strains were incubated in nutrient broth at 37 °C to an optical density of 0.5 McFarland turbidity standards. This suspension was inoculated onto a Mueller-Hinton Agar plate by swabbing with sterile cotton swabs. Two antimicrobial disks were placed 30 mm apart (Centre to Centre). One of the disks contained amoxicillin/clavulanic acid and the other contained an expanded-spectrum cephalosporin (ceftriaxone).

3. Results

3.1. Determination of extra cellular enzymes production

Determination of extra cellular enzymes production of bacterial isolates recovered from urine samples of HIV seropositive pregnant women were carried out for their ability to produce certain enzyme. Out of fifty bacterial isolates, fifteen of *Staphylococcus aureus*, one of *Escherichia coli* and five of *Pseudomonas* spp were positive for lipase. For protease screening, twelve of *Staphylococcus aureus* and five of *Pseudomonas* spp were positive. While twenty of *Staphylococcus aureus*, five of *Pseudomonas* spp and one of *Escherichia coli* were positive for DNase screening.

3.2. Screening for extended spectrum beta lactamase (ESBL)

This was done by using double disk approximation method [13]. Four out of ten *Escherichia coli* and two out of twenty *Staphylococcus aureus* were positive for ESBL.

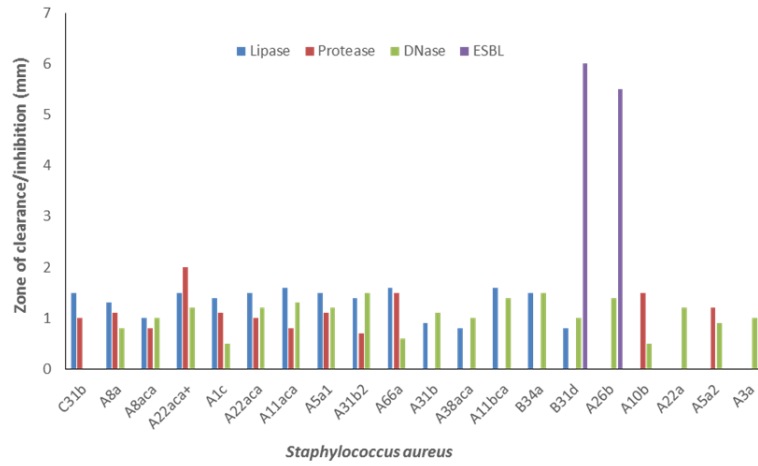


Figure 1 Extracellular enzymes produced by *S. aureus* isolates

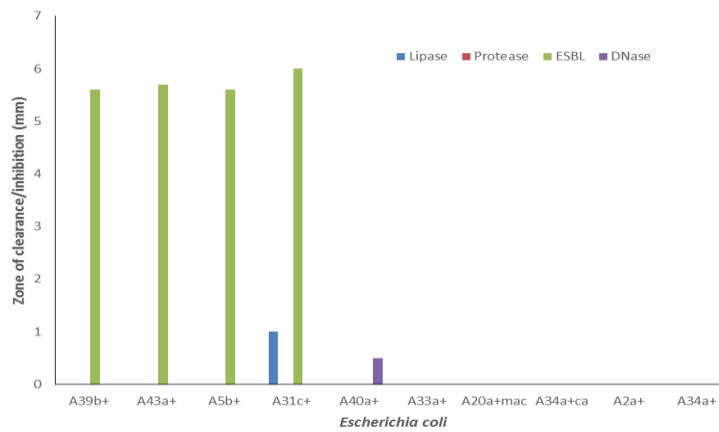


Figure 2 Extracellular enzymes produced by *E. coli* isolates

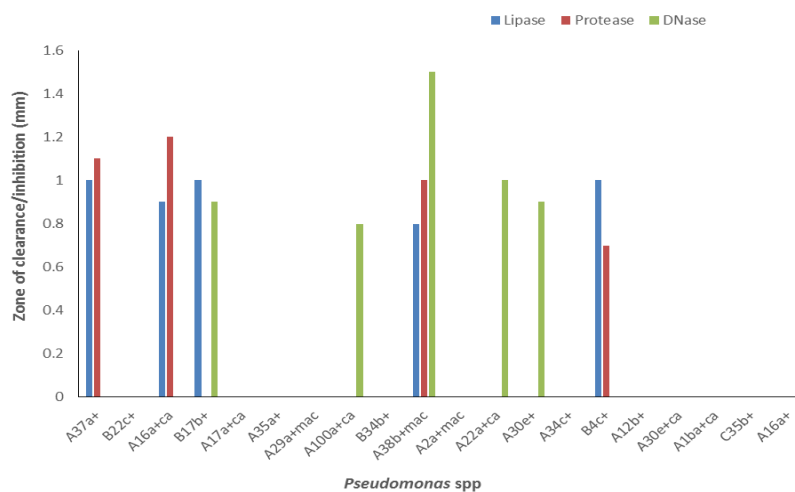


Figure 3 Extracellular enzymes produced by *P. aeruginosa* and *P. fluorescens* isolates

4. Discussion

Enzymes elaborate by organisms are used to inactivate and destroy antibiotics. The previous study revealed that the production of enzyme is both constitutive and inductive which suggests that the enzyme could be an intrinsic defense

of the organisms against the effect of antibiotics used. The bacterial isolates were screened for the production of enzyme protease, lipase, and DNase. Of the 20 *S. aureus* isolates tested, ten (10) isolates were found to produce three of these enzymes each (protease, lipase, and DNase), five bacterial isolates produced DNase and lipase enzymes, two bacterial isolates produced DNase and protease while the remaining three bacterial isolates produced DNase enzyme only. Ten *E. coli* isolates screened for enzyme production showed that one bacterial isolate produced DNase enzyme, another bacterial isolate produced lipase and another protease. Also, of the twenty *Pseudomonas* spp cultured, (13 *Pseudomonas aeruginosa* and 7 *Pseudomonas fluorescens*) 6(46.2%) out of 13 *P. aeruginosa* isolates produced enzymes. Two of which produced lipase and protease each, one produced lipase and DNase while the remaining three produced DNase only. On the other hand, 2(28.6%) out of 7 *P. fluorescens* isolates recovered showed that one of the two *P. fluorescens* produced lipase and protease and the other one produced three enzymes, lipase, protease and DNase.

Production of extended spectrum beta lactamase (ESBL) was detected in this study. According to Tumane and Durgesh (2013), the ESBL producing bacterial causes various life threatening infections which could lead to sepsis related mortality [14]. Of the ten *E. coli* isolates screened for ESBL, four isolates produced ESBL, while *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* isolates were found to be ESBL negative. Of the twenty *S. aureus* isolates screened for ESBL, two isolates were ESBL positive, this is rear information compared to previous researches done and it may be a new idea or innovation in research area. This study highlights the need to establish an antimicrobial resistance surveillance network for ESBL/extracellular producing bacterial isolates to monitor the trends and new types of resistance mechanisms in Nigeria.

5. Conclusion

The study concluded that most of the bacterial isolates cultured from urine samples of HIV seropositive pregnant women in the study area were found to have these virulence factors. Also, the enzyme screening revealed that most of the enzyme-producing bacteria produced more than one analyzed enzyme. Finally, the study recommend that the factors responsible for the selection and dissemination of ESBL-producing strains need to be identified, controlled and where possible, to prevent in order to avoid major outbreaks of such strains in the country.

Compliance with ethical standards

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Disclosure of conflict of interest

None declared.

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