ORIGINAL ARTICLE

Susceptibility of Clinical Isolates to Ceftriaxone-Sulbactam at the Ghana Police Hospital

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ABSTRACT

Background: Ceftriaxone is the most common and widely used third generation cephalosporin empirically. Extensive use of ceftriaxone makes its highly prone to misutilization with increasing reports of microbial resistance globally, which sometimes linked to production of beta-lactamase. Combination therapy of ceftriaxone with sulbactam, a beta-lactamase inhibitor preserves its antibacterial activity. Periodic local antimicrobial surveillance of antibiotics is necessary to enhance their rational prescribing. **Aim:** The study aimed at determining susceptibility pattern of clinical isolates from various specimens to ceftriaxone-sulbactam at the Ghana Police Hospital. **Materials and Methods:** Various types of clinical specimen (n=163) from patients were collected from October 2015 to February 2016. Microorganisms were identified after initial culturing at 37°C for 24 hours. Susceptibility of isolated pathogens to ceftriaxone-sulbactam at a break point of 30/15 µg was done using Kirby-Bauer disc diffusion method. Descriptive statistics was used to analyse data. **Results:** Most prevalent isolates were *Coliforms* (29.4%), *Staphylococcus aureus* (27%) and *E. coli* (17.2%). Overall susceptibility of isolates from the various specimens was 96.4%. Isolates obtained from urine, urethra, sputum and wound specimens showed overall susceptibility rates of 95.7%, 95.2%, 90% and 90% respectively, whilst isolates from the remaining specimens showed 100%. **Conclusion:** Study revealed high microbial susceptibility to ceftriaxone-sulbactam. Thus, this combination therapy offers a cost-effective alternative in the management of infections which ceftriaxone is recommended.

Key words: Susceptibility pattern, ceftriaxone-sulbactam, clinical isolates

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INTRODUCTION

Antimicrobial resistance still remains a major global public health concern. The increasing rate of microbial resistance in health facilities and community settings worldwide can be attributed to a number of factors including overuse, misuse and abuse of antibiotics.[1,2] The consequence of these attitudes by prescribers and patients includes increased; morbidity, complication of infections, treatment cost and mortality in some instances.[3,4] Due to the prevailing penicillin resistance in many countries, broad spectrum cephalosporin's are often used in developing countries to treat most infections.

Ceftriaxone is a broad spectrum third generation beta-lactam antibiotic introduced in the 1980s. It is claimed to be the most common third generation cephalosporin and is widely used empirically because of its safety profile, favourable pharmacokinetic profile which allows once or twice daily dosing. However, its broad spectrum of activity, relatively low cost and availability in many countries, coupled with its extensive use over the years makes it highly prone to misutilization and increased microbial resistance.[5,6] A national antibacterial resistance surveillance of the Chinese Ministry of Health revealed high resistance ranging from 35-70% in majority of isolates with the exception of *Streptococcus spp, Proteus mirabilis* and *Salmonella spp* to ceftriaxone.[7] In a one-year antimicrobial drugs resistance study conducted between December 2002 to December 2003 in Ghana, resistance to ceftriaxone was found to be only 6.3%.[8] Recent laboratory-based nationwide surveillance of antimicrobial resistance in Ghana however revealed that, resistance to ceftriaxone was more than 50%.[9] Production of beta-lactamase which destroys penicillin's and cephalosporin's by hydrolysing their beta-lactam nucleus, is the most common mechanism of resistance by many microbes. This compelled the global scientific community to explore alternative methods of solving this challenge. Among the proposed novel antimicrobial resistance strategies, involved the use of combination of beta-lactam antibiotics with beta-lactamase inhibitors such as sulbactam, tazobactam and clavunalic acid to maintain the potency of beta-lactam antibiotics. Beta-lactamase inhibitors also possess minimal or no antibacterial activity, and they tend to protect the antibacterial effect of some beta-lactam antibiotics when presented as a combination therapy. Although reports have showed that, some beta-lactamase inhibitors particularly cluvalanate can induce autogonism in some beta-lactamases through inducing their production in microbes, such an activity has not been observed with sulbactam.[10,11] Sulbactam is also an irreversible inhibitor of beta-lactamase. Hence, it binds to the enzyme and inhibits their destructive hydrolytic activity to interact with most common forms of beta-lactam antibiotics to maintain their potency and effectiveness in treating infections.[12,13] Sulbactam combinations are widely used for several clinical purposes due to their *in-vitro* activity against several aerobic and anaerobic bacteria, as well as exhibiting reliable pharmacokinetic and safety profiles.[12] Though ceftriaxone-sulbactam has been used in many parts of the world, and recently in Ghana, no microbial susceptibility tests have been conducted in the country to the best of our knowledge. This study sought to determine the antibiotic susceptibility pattern of clinical isolates from various specimens to ceftriaxone-sulbactam at the Ghana Police hospital. Findings would provide local and reference susceptibility data for prescribing rationally at the hospital, and other parts of the country.

MATERIALS AND METHODS

Study population

This descriptive cross-sectional study involved examination of various types of samples (163) obtained from both inpatients and outpatients who visited the Ghana Police Hospital's Microbiology Laboratory from October 2015 to February 2016. The protocol for this study was approved by the Ghana Police Hospital Administration.

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Sample collection and identification of isolates

Various types of clinical specimen of patients; urine, vaginal, urethral, sputum, wound, blood, ear and eye were aseptically obtained at the Ghana Police Hospital laboratory. Only non-repetitive specimens with isolates which were identified during the study period were included. Isolated microbial colonies were identified using morphological features of the colonies, as well as standard biochemical and serological methods.[14] After identification, each colony, representing an isolate was emulsified in 2 ml sterile peptone water, incubated at 37°C for 24 hours, diluted to 0.5 MacFarland turbidity standards, and then transferred into sensitivity agar plates (Biotec Laboratories, UK).

Susceptibility testing

Ceftriaxone-sulbactam sensitivity discs used in this study were stored between -2 to 8°C in a refrigerator to maintain its sensitivity, until it was used for examination. Antimicrobial susceptibility testing of isolated microbes was done using the Kirby-Bauer disc diffusion method[15], against ceftriaxone-sulbactam with a break point of 30/15 µg [Himedia Laboratory PVT Ltd, Mumbai-India]. The discs were placed on agar plates pre-inoculated with isolated test organisms. The plates were incubated at 37°C for 24 hours after which the lytic zones were measured. Inhibition zone diameters pertaining to ceftriaxone-sulbactam were measured using callipers and compared with standard interpretation charts[16], and scored as sensitive or resistant.

DATA ANALYSIS

The laboratory data obtained on patients were checked for completeness, entered, and analysed using Graph Pad Software version 5.0 for windows (Graph Pad software, San Diego California, USA). Descriptive statistics (percentages and frequency) were used to present the findings of the study.

RESULTS

Of the 163 patients within the age ranges of <20, 20-39, 40-59 and ≥ 60 years whose specimens were used in this study, 113 (69.3%) were females and 50 (30.7%) were males as shown in Table 1. The minimum and maximum age of study participants ranged from 1 year to 84 years. The overall mean age and standard error of mean (SEM) of study participants was 33.8 ± 1.3 years, whilst that of males was 36.6 ± 2.2 years compared with 31.0 ± 1.6 years for females.

The various clinical specimens obtained during the study period were from; 69 (42.3%) urine, 41 (25.2%) high vaginal swab (HVS), 21 (12.9%) urethra, 10 (6.1%) sputum, 10 (6.1%) wound, 5 (3.1%) blood, 6 (3.7%) ear and 1 (0.6%) eye (0.6%). Majority of the clinical specimen were obtained from female patients 113 (69.3%) and 94 (57.6%) patients within the age group of 20-39 years.

The most prevalent isolates were *Coliforms* (29.4%), *Staphylococcus aureus* (27%), *E. coli* (17.2%), *Klebsiella spp* (7.4%), *Pseudomonas spp.* (5.5%) and *Proteus mirabilis* (4.3%). These top six isolates together constituted (90.8%). The rest of the isolates were mixed *Candida spp* with *S. aureus, Candida spp.* with *Coliform, Candida* with *E. coli* and *S. aureus* and *N. gonnorrhoeae.* Detailed frequency and percentages of isolates examined during the study period are presented in Table 2.

Further categorization of isolates showed that, gram negative bacteria constituted 105 (63.8%), gram positive 44 (27%), mixed fungal with bacteria 14 (8.6%) and mixed bacteria isolates was 1 (0.6%).

Overall susceptibility of isolates from the various specimens was 157 (96.4%) whilst overall resistance was 3.7%. Gram negative isolates showed overall susceptibility of 98.7% to ceftriaxone-sulbactam, whilst the only gram positive isolate in this study (*Staphylococcus aureus*) showed 95.5% susceptibility. The top three most prevalent isolates *Coliforms, S. aureus* and *E. coli* showed overall susceptibility rates of 93.8%, 95.5% and 96.4% to ceftriaxone-sulbactam in this study. The rest of the isolates (both single and mixed) showed 100% susceptibility to ceftriaxone-sulbactam. The detailed susceptibility and resistance pattern of the clinical isolates are presented in Table 3.

Isolates obtained from urine, urethra, sputum and wound specimens showed overall susceptibility rates of 95.7%, 95.2%, 90% and 90% respectively, whilst isolates from the remaining specimens showed 100% susceptibility to ceftriaxone-sulbactam. The findings revealed that, the mixed fungal and bacteria isolates in this study were observed in only urine samples and high vaginal swabs. Table 4 presents the detailed susceptibility and resistance rates of the isolates to ceftriaxone-sulbactam obtained from the various specimens examined in this study.

DISCUSSION

Microbiological data in this study revealed that, *Coliforms, E. coli, S. aureus, Klebsiella spp, Proteus spp* and *Pseudomonas spp*. were the most prevalent among the various specimens. Our findings were quite similar to observations in various clinical specimens obtained across the regions of Ghana in an antimicrobial resistance study.[8] Furthermore, most of the isolates in this study were gram negative as observed in the latter study. Also, almost all the bacterial isolates in this study were observed in a recent nationwide laboratory-based antimicrobial resistance study in Ghana.[9] However, mixed bacteria and, fungal with bacteria isolates observed in this study were not reported in the latter studies.[8,9] This study observed that, clinical specimens from urine, swabs and blood constituted over 90%, and this was similar to recent findings in Ghana and other studies.[9,17,18] Furthermore, blood and eye specimens constituted the lowest proportion among the various specimens collected and analysed within the study period. In this study, gram negative and positive isolates were identified, and also mixed fungal with bacteria isolates as observed in previous studies conducted in the hospital.[19,20] It is worth noting that, the mixed fungal and bacteria isolates in this study were from urine and HVS specimens, whilst those of the latter studies conducted in the Police hospital were from only urine samples.

Studies have shown that, a once-daily dose of ceftriaxone-sulbactam is effective in the treatment of several infections due to its high plasma-minimum inhibitory levels.[21] A multicentre clinical study in China which administered 2.5 g ceftriaxone-sulbactam twice-daily to patients (285) for 7-14 days revealed cure, effective and bacterial eradication rates of 39.55%, 85.07% and 83.85%, respectively.[22] Another clinical study to assess the efficacy of combination of ceftriaxone, sulbactam and disodium edetate for treatment of multi-drug resistant septicaemia in a tertiary hospital in India revealed clinical cure in terms of relief of symptoms and bacteriological eradication response of 83.3%.[23] With exception of the top three isolates *Coliforms, E. coli and S. aureus* from the various specimens which showed 93.8%, 95.5% and 96.4% sensitivities, the rest of the isolates exhibited 100% susceptibility

Table 1: Age and sex distribution of patients whose specimen was used for the study

Sex	Age groups n (%)				
	<20 years	20-39 years	40-59 years	≥ 60 years	Total
Male	7 (4.3%)	23(14.1%)	14 (8.6%)	6 (3.7%)	50 (30.7%)
Female	20 (12.3%)	71 (43.5%)	15 (9.2%)	7 (4.3%)	113(69.3%)
Total	27(16.6%)	94 (57.6%)	29 (17.8%)	13 (8.0%)	163(100%)

Table 2: Isolates collected from various clinical specimens within the study period

Organisms	Numbers (n=163)	Percentages (%)
Coliforms	48	29.4
S. aureus	44	27.0
E. coli	28	17.2
Klebsiella spp.	12	7.4
Pseudomonas spp.	9	5.5
Proteus mirabilis	7	4.3
Candida spp and *S. aureus*	6	3.7
Candida spp. and *Coliform*	5	3.1
Candida and *E. coli*	3	1.8
S. aureus and *N. gonnorrhoeae*	1	0.6

Table 3: Overall susceptibility pattern of isolates to ceftriaxone-sulbactam

Organisms	Sensitivity n (%)	Resistance n (%)
Coliforms	45(93.8)	3 (6.2)
S. aureus	42 (95.5)	2 (4.5)
E. coli	27 (96.4)	1 (3.6)
Klebsiella spp.	12 (100)	-
Pseudomonas spp.	9 (100)	-
Proteus mirabilis	7 (100)	-
Candida spp and *S. aureus*	6 (100)	-
Candida spp. and *Coliform*	5 (100)	-
Candida spp. and *E. coli*	3 (100)	-
S. aureus and *N. gonnorrhoeae*	1 (100)	-

Table 4: Susceptibility pattern of isolates from various specimens to ceftriaxone-Sulbactam

Organisms	Susceptibility and Resistance rates (%) in various specimens							
	Urine	HVS	Urethra	Sputum	Wound	Blood	Ear	Eye
Coliforms	**S 21** (91%) **R 2**	**S 11** (100%) **R -**	**S 4** (100%) **R -**	**S 3** (100%) **R -**	**S 3** (75%) **R 1**	**S 3** (100%) **R -**	------	------
S. aureus	-------	**S 17** (100%) **R -**	**S 15** (93.8%) **R 1**	**S 3** (75%) **R 1**	**S 3** (100%) **R -**	--------	**S 4** (100%) **R -**	------
E. coli	**S 25** (100%)	**S 2** (100%)	--------	----------	---------	--------	---------	-------
Klebsiella spp.	**R - 1** **S 10** (100%)	**R -** -------	---------	**S 1** **(100%)**	**S 1** **(100%)**	---------	---------	-------
Pseudomonas spp.	**R -** **S 3** (100%)	-------	--------	**R -** **S 2** (100%)	**R -** --------	**S 2** (100%)	**S 1** (100%)	**S 1** (100%)
Proteus mirabilis	**R -** **S 4** (100%)	--------	--------	--------	**S 2** (100%) **R -**	--------	**S 1** (100%) **R -**	-------

Candida spp and *S. aureus*	------	**S 6** (100%) **R -**	--------	---------	---------	--------	--------	------
Candida spp. and *Coliform*	--------	**S 5** (100%) **R -**	--------	---------	---------	--------	-------	-------
Candida and *E. coli*	**S 3 (100%) R -**	-------	---------	---------	---------	--------	-------	--------
S. aureus and *N. gonnorrhoeae*	--------	-------	**S 1** (100 %) **R -**	---------	---------	--------	-------	--------
Overall (%)	**S 95.7**	**S 100**	**S 95.2**	**S 90**	**S 90**	**S 100**	**S 100**	**S 100**
	R 4.3	**R -**	**R 4.8**	**R 10**	**R 10**	**R -**	**R -**	**R -**
Total specimens	**69**	**41**	**21**	**10**	**10**	**5**	**6**	**1**

Nil: -----/-

to ceftriaxone-sulbactam. Further *in vitro* analysis of the efficacy ceftriaxone-sulbactam against isolates in the various specimens revealed that, with exception of isolates from urine, urethra, sputum and wound which showed overall susceptibility of 95.7%, 95.2%, 90% and 90% respectively, 100% susceptibility rates was observed with the rest of the isolates from the other specimens. Findings from this aspect of the study suggest high efficacy of ceftriaxone-sulbactam *in vitro* and tends to agree with the high efficacy rates observed in the latter clinical studies reported.[21-23] Furthermore, the overall 96.4% susceptibility of isolates to ceftriaxone-sulbactam observed in this study was greater than the over 50% resistance to ceftriaxone seen among microbial isolates in a recent study conducted across regions in Ghana.[9] Extended-broad spectrum beta-lactamases have been identified as contributing to antimicrobial resistance to ceftriaxone in Ghana,[24] and parts of Africa.[25] Hence, the high microbial susceptibility rates to ceftriaxone-sulbactam seen in this study may be attributed to the fact that, this combination of antibiotic is relatively new on the Ghanaian pharmaceutical market and may not have been misused since its introduction. Also, the synergistic effect of sulbactam to ceftriaxone due to its weak antibacterial activity coupled with its inhibitory beta-lactamase activity certainly might have contributed to the high susceptibility rates of isolates to ceftriaxone in this study than observed in the recent nationwide study.[9] It is also worth noting that, a lower overall microbial resistance of 3.4% to ceftriaxone-sulbactam was observed in this study than the 6.3% seen in the nationwide microbial resistance to only ceftriaxone in the study by Newman *et al.*[8]

A limitation of this study was the small total sample size of the various types of specimen used for the analysis. This was due to the limited ceftriaxone-sulbactam discs which were available for the study. Another limitation of this study was our inability to conduct a simultaneous antimicrobial test between ceftriaxone-sulbactam and ceftriaxone to provide comparative *in vitro* efficacy results. However, to the best of our knowledge, this is the first antimicrobial susceptibility test against ceftriaxone-sulbactam in Ghana.

CONCLUSION

Considering the evidence that, almost all of the clinical isolates showed very high susceptibility to ceftriaxone-sulbactam *in vitro*, it tends to suggest that, it would be a better cost-effective alternative in the management of infections which ceftriaxone is recommended. Consequently, a nation-wide antimicrobial susceptibility surveillance to ceftriaxone-sulbactam is also recommended to confirm our observation or otherwise. Further *in vivo* study in patients is suggested to provide evidence-based clinical data of its efficacy in our setting for prescribers in Ghana.

Disclosure

Authors declare there are no conflicts of interest.

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