



Evaluations of some Serum Biochemical Parameters and Antibiotics Susceptibility Pattern in *Enterococcus faecalis* Infected Patients

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Abstract

The evaluations of some serum biochemical parameters and antibiotics susceptibility pattern in *Enterococcus faecalis* infected patients were studied. Early morning midstream urine and blood specimens were collected from thirty *Enterococcus faecalis* infected patients aged 18-35 years and from another thirty subjects with no evidence of infection (control) aged 18-35 years. The following serum biochemical parameters: creatinine, total protein, albumin, globulin, C-reactive protein, sodium, potassium, chloride and bicarbonate were quantitatively measured while antibiotics susceptibility test was performed on the pure isolate of *Enterococcus faecalis* obtained from the cultured urine specimen using the disc diffusion method. The results of this study showed that there was significant difference of serum C-reactive protein in the *Enterococcus faecalis* infected patients as compared with that of the control subjects while there were no significant differences in the other serum biochemical parameters of the *Enterococcus faecalis* infected patients as compared with that of the control subjects. However, the antibiotics susceptibility results showed that only two antibiotics: pefloxacin and ciprofloxacin had considerable susceptibility of 56.67% and 66.69% respectively while others showed very poor susceptibility as follow: ofloxacin 46.67%, gentamycin 36.67%, ampiclox 23.33%, erythromycin 33.33%, norfloxacin 20%, chloramphenicol 16.67%, clindamycin 20% and streptomycin 26.67%. In conclusion, this study has revealed that serum C-reactive protein concentration is significantly elevated in *Enterococcus faecalis* infected patients and ciprofloxacin among the several antibiotics used for this research work has proved to be the most susceptible antibiotic for the empirical treatment of this infection in the studied community followed by pefloxacin.

Keywords: *Enterococcus faecalis* infection, Serum Biochemical Parameters, Antibiotics Susceptibility, Evaluation.

Introduction

Enterococci are non-spore forming and non-motile (except for an occasional flagellated strain) microorganisms that are capsulated with slimy layers. They are facultative anaerobes that have the ability to ferment many sugars with the subsequent production of lactic acid. The colonies are usually small, not pigmented and glistening. The genus *Enterococcus* include large and different group of bacteria with some of them been normal residents or agents of disease in human beings as well as in animals while others live freely in the environment. Members of this group which are one of the commonest among bacterial infections are arranged in cocci, bead-like chains which are found in pairs with varying length¹.

Antibiotic susceptibility is the response of a patient's organism to the antibiotic(s) of choice in the course of treatment provided the recommended dosage is adhered to as reported by Nijs et al. 2000².

Urinary tract is a complicated tube which is lined with epithelial

cells and opened to the body surface, a situation which makes it easier to be invaded by organisms that are exogenous. It is considered as one of the commonest bacterial infections³. About 5% of adults that are young are known to be infected with bacteria at one point in time, while approximately 50% of adult women are known to have urinary tract infection at sometime in their life with the occurrence of this infection increasing with age at the rate of 1-2% per decade as reported by Bishop in his research work of uncomplicated urinary tract infections⁴ while Hooton reported recurrence in 27-48% of healthy women⁵. However, Foxman et al. also reported recurrence even after treatment with antibiotics⁶.

For several years, studies have shown antibiotics treatment of choice (susceptibility) for some microorganisms associated with urinary tract infections, but rarely has such studies been carried out on *Enterococcus faecalis* which is becoming extremely resistant to antibiotics within the studied community. Besides, documented studies on the effects of this microorganism (*Enterococcus faecalis*) on serum biochemical parameters are

not available thus the need for this research work which is aimed at the evaluation of some serum biochemical parameters and antibiotics susceptibility pattern in *Enterococcus faecalis* infected patients.

Materials and Methods

A total of 30 patients aged 18-35 years, attending out patients clinics in some hospitals in Yenagoa, Bayelsa state of Nigeria whose early morning midstream urine specimens upon culture grew ≥ 10 cfu/ml pure growth of *Enterococcus faecalis* as well as another 30 subjects aged 18-35 years who were free from infections generally (control) constituted the study population of this work.

After seeking the consent and approval from these patients/control subjects, early morning midstream urine specimens as well as 5ml blood specimens were collected from each of them into sterile universal bottles and non anticoagulated bottles respectively. The urine specimens were cultured for bacterial growth according to standard procedure, using cystine lactose electrolytes deficient (CLED) agar and incubated aerobically at 37°C with the results read at 24 hours of incubation. Antibiotics susceptibility test was carried out on the pure isolate of the *Enterococcus faecalis* via the use of disc diffusion method as described by Ezeonu and Ugwu 2010⁷. The inhibition zone diameter was interpreted as either resistant or susceptible. A diagnosis of antibiotic susceptibility was made if ≥ 17 mm zone diameter was obtained and resistant if ≤ 11 mm zone diameter was obtained. The blood specimens which were collected into non anticoagulated bottles were allowed to clot, carefully retracted, spun and the sera obtained used for the quantitative measurement of the following biochemical parameters using S23A model spectrophotometer: Creatinine, Jaffe reaction method as described by Henry in Textbook of Clinical Chemistry and modified by Randox Laboratory kit manual⁸, total protein, Biuret method as described by Weichselbaum and modified by Randox Laboratory kit manual⁹, albumin, Bromocresol green method as described by Doumas et al. 2004 and modified by Randox Laboratory kit manual¹⁰, globulin, Sandle and Alderson method as described by Cynthia et al. 1993 in Textbook of Laboratory Test and Diagnostic Procedure, 2nd edition, W.B. Saunders Company, Philadelphia¹¹ C-reactive protein as described by Kari in Spin react Diagnostic kit manual, Spain¹², sodium as described by Tietz in Teco Diagnostic kit manual, Anaheim, California¹³, potassium as described by Terri in Teco Diagnostic kit manual, California¹⁴, chloride as described by Young in Teco Diagnostic kit manual, Anaheim, California¹⁵ and bicarbonate as described by Ochei and Kolhatkar in Textbook of Medical Laboratory Science, Theory and Practical, 8th edition, Tata Mc Graw-Hill Publishing Company Limited, New Delhi¹⁶.

Statistical Analysis: The results of this study were expressed in mean and standard deviation, while the differences between the

subjects (control and infected) were assessed using the Student's "t" test. The results were considered statistically significant at $p < 0.05$

Results and Discussion

In this study, the mean values of the estimated serum biochemical parameters in the *Enterococcus faecalis* infected patients were compared with that of the control subjects while the antibiotics susceptibility pattern of the *Enterococcus faecalis* infected patients was evaluated

A summary of the results of serum biochemical parameters in the patients infected with *Enterococcus faecalis* as compared with that of the control subjects is shown in Table-1 while Table-2 shows the percentage of antibiotics susceptibility pattern of the *Enterococcus faecalis* infected patients.

The results of the mean serum C-reactive protein concentration in the *Enterococcus faecalis* infected patients was significantly higher ($p < 0.05$) than that of the control subjects as confirmed in this study. It is presumed that the cause of this elevated C-reactive protein concentration may be due to inflammation within the urinary tract of these patients which is indicative of an invasive *Enterococcus faecalis* infection that requires appropriate treatment.

The mean serum creatinine concentration in the *Enterococcus faecalis* infected patients showed no statistically significant difference ($p > 0.05$) as compared with that of the control subjects. This finding which is confirmed in this study has proved that *Enterococcus faecalis* infection does not have any effect on the serum concentration of creatinine.

The mean serum total protein concentration in the *Enterococcus faecalis* infected patients showed no statistically significant difference ($p > 0.05$) as compared with that of the control subjects. It is pertinent to report that this finding which is confirmed in this study is suggestive that *Enterococcus faecalis* infection has no effect on serum total protein concentration.

The difference between the mean serum albumin concentration of the *Enterococcus faecalis* infected patients as compared with that of the control subjects showed no statistical significance ($p > 0.05$) an indication showing that *Enterococcus faecalis* infection has no effect on the serum concentration of this biochemical parameter.

The results of this study went further to show that the mean serum globulin concentration in the *Enterococcus faecalis* infected patients was not significantly higher ($p > 0.05$) as compared with that of the control subjects. This finding which is confirmed in this study as shown that this infection has no effect on the serum concentration of this biochemical parameter.

Table-1
Results of the Biochemical Parameters for those infected by *Enterococcus faecalis* compared with the control subjects

Parameters measured	Control (n=30)	Infected (n=30)	Remark
Creatinine (mg/dl)	0.77 ± 0.13	0.78 ± 0.14	NS
Total protein (g/l)	71.20 ± 4.75	71.60 ± 5.00	NS
Albumin (g/l)	40.08 ± 1.41	40.50 ± 1.52	NS
Globulin (g/l)	31.12 ± 4.25	31.10 ± 4.11	NS
C-reactive protein (mg/l)	0.23 ± 0.05	10.17 ± 3.38	S
Sodium (mEq/l)	141.16 ± 3.64	141.30 ± 4.00	NS
Potassium (mEq/l)	4.02 ± 0.35	4.05 ± 0.38	NS
Chloride (mEq/l)	100.00 ± 1.85	100.40 ± 1.87	NS
Bicarbonate (mmol/l)	25.70 ± 1.31	25.74 ± 1.40	NS

Values are mean and S.D. of duplicate determinations at p< 0.05, NS - represents not significant, S -represents significant, n - represents number of subjects.

Table-2
Percentage of antibiotics susceptibility pattern in the *Enterococcus faecalis* infected patients

Antibiotics	Antibiotics Concentrations	N	Antibiotics Susceptibility	Antibiotics Resistance
OFL	5 (µg)	30	(14)46.67%	(16)53.33%
PFL	10 (µg)	30	(17)56.67%	(13)43.33%
GNT	10 (µg)	30	(11)36.67%	(19)63.33%
AMP	20 (µg)	30	(7)23.33%	(23)76.67%
CPF	5 (µg)	30	(20)66.67%	(10)33.33%
ERY	10 (µg)	30	(10)33.33%	(20)66.67%
NFX	10 (µg)	30	(6)20.00%	(24)80.00%
CHL	30 (µg)	30	(5)16.67%	(25)83.33%
CLN	10 (µg)	30	(6)20.00%	(24)80.00%
STR	10 (µg)	30	(8)26.67%	(22)73.33%

a: The number of patients that are sensitive and resistant to antibiotics are in parenthesis while their values are in percentage b: N is number of subjects c: OFL is *ofloxacin* d: PFL is *pefloxacin* e: GNT is *gentamycin* f: AMP is *ampiclox* g: CPF is *ciprofloxacin* h: ERY is *erythromycin* i: NFX is *norfloxacin* j: CHL is *chloramphenicol* k: CLN is *clindamycin* l: STR is *streptomycin*

The levels of electrolytes in the blood are usually the outcome of fine regulatory mechanisms of ionic charges and osmotic balance of the extracellular fluid which involves the kidneys, lungs and the endocrine system. In this study, the serum

electrolytes (sodium, potassium, chloride and bicarbonate) levels in the *Enterococcus faecalis* infected patients as compared with that of the control subjects showed no statistical significant differences (p>0.05). This finding which is

confirmed in this study is an indication that the levels of electrolytes in the blood are usually not elevated in *Enterococcus faecalis* infected patients.

However, the results of the antibiotics susceptibility in patients infected with *Enterococcus faecalis* within the studied community showed that among the several antibiotics used for this research work only two were considerably significant and these were pefloxacin with a percentage susceptibility of 56.67% and ciprofloxacin with a percentage susceptibility of 66.67%. All the other antibiotics showed very poor susceptibility as follows: ofloxacin 46.67%, gentamycin 36.67%, ampiclox 23.33%, erythromycin 33.33%, norfloxacin 20.00%, chloramphenicol 16.67%, clindamycin 20% and streptomycin 26.67%.

Ofloxacin is an antibiotic which belongs to the fluoroquinolone drug class. It is an antibiotic which is chemotherapeutically synthetic in nature as well as being the second among the fluoroquinolone drugs regarding penetration as reported by Nelson et al.¹⁷. The result from this study revealed that fourteen of the patients infected with *Enterococcus faecalis* which accounted for 46.67% demonstrated susceptibility to ofloxacin while sixteen of these infected patients which accounted for 53.33% were resistant to ofloxacin. This is a finding confirmed in this study and is in agreement with the research work of Gledrys and Bilska who compared the susceptibility of bacteria isolated from various clinical materials between 1989-1991 to ofloxacin and found out that *Staphylococcus aureus*, *Escherichia coli*, *Proteus*, *klebsiella* and *Enterobacter* were frequently susceptible to the antibiotic while a gradual increase in resistance was noted in *Enterococcus faecalis*¹⁸.

It is presumed from this study that the weak susceptibility rate of ofloxacin to the *Enterococcus faecalis* isolated from these patients may be associated with its fair ability to inhibit the A subunit of *deoxyribonucleic acid gyrase* which is essential in the reproduction of bacterial *deoxyribonucleic acid* while the gradual increase in resistance of this microorganism to ofloxacin as confirmed in this work may be suggestive of its ability to withstand the effects of this antibiotic a situation which therefore calls for further research work in order to unfold how this microorganism is able to do this.

This study showed that seventeen patients infected with *Enterococcus faecalis* thus accounting for 56.67% were susceptible to pefloxacin while thirteen of these infected patients accounting for 43.33% were resistant to pefloxacin. This finding as confirmed in this study may be due to the ability of the pefloxacin to inhibit the A subunit of *deoxyribonucleic acid gyrase* which is essential in the reproduction of bacterial *deoxyribonucleic acid* to a reasonable extent.

The result of ciprofloxacin, a broad spectrum fluoroquinolone antibacterial agent showed that twenty of the patients infected with *Enterococcus faecalis* accounting for 66.67% were

susceptible, while ten of them accounting for 33.33% were resistant. This finding which is confirmed in this study agrees with that of Wiseman¹⁹, who in his research work of an updated review of ciprofloxacin pharmacology, therapeutic efficacy and tolerability reported that ciprofloxacin is effective in the treatment of a wide variety of infections. This may be due to its ability to inhibit vigorously the A subunit of *deoxyribonucleic acid gyrase* which is essential in the reproduction of bacterial *deoxyribonucleic acid*

However, the poor rate of antibiotics susceptibility to *Enterococcus faecalis* as confirmed in this study: ofloxacin 46.67%, gentamycin 36.67%, ampiclox 23.33%, erythromycin 33.33%, norfloxacin 20%, chloramphenicol 16.67%, clindamycin 20% and streptomycin 26.67% may be attributed to the high level of antibiotics abuse arising from self medication, inadequate dosage or over dosage.

Conclusion

In conclusion, serum C- reactive proteins concentration is elevated in *Enterococcus faecalis* infected patients with ciprofloxacin being the most susceptible antibiotic for the empirical treatment of this infection in the studied community followed by pefloxacin.

Recommendation: It is recommended that: i. Serum C-reactive protein concentration should be measured in all *Enterococcus faecalis* infected patients and this concentration should be brought to normal level upon the finding of any significant elevation during treatment.

Ciprofloxacin should be used mostly for the empirical treatment of *Enterococcus faecalis* infection in the studied community followed by pefloxacin as an alternative antibiotic in a situation where ciprofloxacin is not readily available.

The susceptibility of microorganisms to antibiotics should be reviewed from time to time in every community in order to give update information on antibiotics susceptibility of various microorganisms which in turn would drastically reduce the scourge of antibiotics resistance.

Public awareness campaigns should be embarked upon by appropriate authorities to enlighten the public on the need to desist from antibiotics abuse.

Physicians should always refer suspected cases of urinary tract infection(s) to a registered and licensed Medical Laboratory Facility for urine microscopy, culture and sensitivity (m/c/s) in order to confirm the specific bacterium responsible for the infection and recommendation of the most susceptible antibiotic(s) for the treatment of the bacterium. This result when combined with clinical information and experience would go a long way in enhancing effective treatment.

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