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DETECTION OF ESBL, AMP C AND ITS SIGNIFICANT ASSOCIATION WITH BIOFILM PRODUCTION IN NON-FERMENTING GRAM NEGATIVE BACILLI

Microbiology

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ABSTRACT

A total of 122 non fermenting gram negative bacilli isolated from various clinical samples were selected for the study. Isolates which were resistant to third generation cephalosporins selected and tested for ESBL and Amp C production. All the isolates were also tested for biofilm production by tube adherence method. Analyse both to check whether any correlation between these two parameters. Of total 122 isolates of nonfermentors studied Pseudomonas aeruginosa (58.3%) was the commonly isolated and 30% were biofilm producers. Among 122 isolates, 14.8% were ESBL producers and 52.5% were AmpC producers. Isolates producing both ESBL and Biofilm were exhibiting more resistance against common classes of antibiotics than the isolates producing ESBL alone, which proves that there is a significant correlation between ESBL production and biofilm production by Non fermenters.

KEYWORDS

Nonfermentors, Antibiotic resistance, Biofilm, Virulence

Introduction

Nonfermenters are now alarmingly increased as pathogens. Drug resistance have been a global issue for last few decades. There are certain other virulent factors also coexist to drug resistance to increase the virulence nature of bacteria. One of the virulent factors is biofilm production, which was noticed in many pathogens.

The aim of this study is to identify the prevalence of ESBL, Amp C and Biofilm production in Nonfermenting gram negative bacilli and to find any correlation between drug resistance and biofilm production.

Materials & Methods

This prospective analytical study included a total of 122 Nonfermenting gram negative bacilli isolated from various clinical samples. Identification of isolates were done by conventional methods.[1] Antimicrobial susceptibility testing was performed as per standard guidelines of CLSI.[2] Isolates which were resistant to third generation cephalosporins selected and tested for ESBL and Amp C production. All the isolates were also tested for biofilm production.

Detection of ESBL production

ESBL production detected by using both double disc synergy (Amoxyclav, Ceftazidime) and combined disc (Ceftazidime + Clavulanic acid).Enhancement of zone of inhibition for ceftazidime which was placed 17 mm away from Amoxyclav disc was considered for ESBL production. In case of combined disc method, increase in zone of inhibition for more than 5mm in combined disc than Ceftazidime disc was alone was also considered for ESBL production.[3]

Detection of Amp C production

Amp C detection was done by two methods.

a) Screening for AmpC β -lactamase

Screening for the inducible AmpC β -lactamase was done by the disc antagonism test[4] by placing cefoxitin disc (30 µg) at a distance of 20 mm from ceftazidime (30 µg) on the surface of MHA. Isolates which showed blunting of the ceftazidime zone adjacent to cefoxitin disc were considered as "screen positive" and selected for AmpC β lactamases detection.

b) Detection of AmpC β lactamases by AmpC Disk Test[5]

Here, a lawn culture of *Escherichia coli* ATCC 25922 was prepared on MHA plate. Sterile disks (6 mm) were moistened with sterile saline (20 µl) and inoculated with several colonies of test organism. The inoculated disk was then placed beside a cefoxitin disk (almost touching) on the inoculated plate. The plates were incubated overnight at 35°C. Appearance of flattening or indentation of the cefoxitin inhibition zone near the test disk is positive. A negative test will have an undistorted zone.

Detection of Biofilm Production

***Tube adherence method*:**

Biofilm production was estimated qualitatively for all the isolates by tube adherence method by Christensen et al[6] Suspension of tested strains was incubated in the glass tubes containing Brain Heart Infusion Broth (broth) aerobically at the temperature of 35°C for the period of 2 days. Then the supernatant discarded and the glass tube was stained by 0.1% safranin solution, washed with distilled water three times and dried. A positive result is defined as the presence of a layer of stained material adhered to the inner wall of the tubes. The exclusive observation of a stained ring at the liquid-air interface should be considered negative.

Result

A total of 122 isolates of non-fermenting gram negative bacilli have been included in this study. Among which *Pseudomonas aeruginosa* (58.3%) and *Acinetobacter baumanni* (28%) were the common isolates, followed by other NFGNB (8.3%) and *Stenotrophomonas spp*(3.3%)(Table 1)

Table 1: Total number(%) of isolates of Non fermentors

Isolate	Number(%)
Pseudomonas aeruginosa	71(58.2%)
Acinetobacter baumanni	37(30.3%)
Other NFGNB	10(8.2%)
Stenotrophomonas spp	4(3.3%)
Total	122

In Figure 1, percentage of biofilm producers among 122 isolates were 36(30%) and biofilm non producers were 86(70%).

Figure 1: Percentage of Biofilm producers and non-Biofilm producers among NFGNB

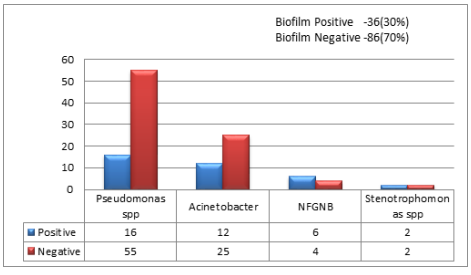

Table 2: Detection of ESBL and AmpC among NFGNB (n=122)

Isolate	ESBL		AmpC		Total
	Positive	Negative	Positive	Negative	
Pseudomonas spp	10	61	43	28	71
Acinetobacter	8	29	19	18	37

NFGNB	0	10	2	8	10
Stenotrophomonas spp	0	4	0	4	4
Total	**18 (14.8%)**	**104 (85.2%)**	**64 (52.5%)**	**58 (47.5%)**	**122**

Of total 122 isolates of nonfermentors studied for AmpC production, 52.5% were AmpC producers and 47.5% were negative for AmpC production. Among 122 isolates,14.8% were positive and 85.2% were negative for ESBL production(Table 2)..

Table 3: Correlation between ESBL and Biofilm producers among NFGNB (n=122)

Organism	Statistical Significance	ESBL producers	ESBL Non Producers	Significance
NFGNB	**Biofilm Positive(36)**	10(55.6%)	26(25%)	0.008
	Biofilm Negative (86)	8(44.4%)	78(75%)	

In table 3, correlation between ESBL and Biofilm producers showed 55.6% of ESBL producers are biofilm positive and 25% of ESBL non-producers are biofilm negative. The chi-square statistic is 6.8872. The *p*-value is .008681. This result is significant at $p < .05$.

Table 4:Correlation between AmpC and Biofilm producers among NFGNB (n=122)

Organism	Statistical Significance	AmpC producers	AmpC Non Producers	Significance
NFGNB	**Biofilm Positive(36)**	21(32.8%)	15(25.9%)	0.7(Not significant)
	Biofilm Negative (86)	43(67.2%)	43(74.1%)	

In table 4, correlation between AmpC and Biofilm producers showed 32.8% of AmpC producers are biofilm positive and 25.9% of AmpC non-producers are biofilm negative. The *p*-value is is 0.7066.. This result is *not* significant at $p < .05$.

Discussion

In our study, *Pseudomonas aeruginosa* (58.3%) and *Acinetobacter baumanni* (28%) were the common isolates, followed by other NFGNB(8.3%) and *Stenotrophomonas spp*(3.3%)similar to a study by Benachinmardi et al,[7] where they also isolated Pseudomonas as the predominant organisms followed by *Acinetobacter spp.*

Of total 122 isolates of nonfermentors studied for AmpC production, 52.5% were AmpC producers and 47.5% were negative for AmpC production. Among 122 isolates, 14.8% were positive and 85.2% were negative for ESBL production well comparable to study by Gupta R et al[8] where they reported 21.4% and 51.1% ffor ESBL and AmpC production. Study by Aggarwal et al [9] revealed that 24.3 per cent of NFGNB were ESBL producers. For AmpC production similar results of 51.4% were reported by Bhattacharjee et al[10]

Biofilm production and presence of ESBL enzymes was significantly correlated in non-fermenting gram-negative bacilli in our study. However, the association between AmpC and biofilm production was not statistically significant in our study. Study by Singhai et al[11],showed there was significant association between MBL production and biofilm producers.

In our study,30% were biofilm producers ,there are studies showing higher percentage of biofilm production than our study. Biofilm growth is associated with an increased level of mutations as well as with quorum-sensing-regulated mechanisms. Antimicrobial resistance is an innate feature of bacterial biofilms.[12] Many studies have shown that biofilm formation is higher in MDR strains. In this study, among 35 isolates, 21 (60.4 %) were biofilm producers.

Conclusion

The present study showed a higher rate of biofilm forming strains among ESBL Producers than non-producers.Isolates producing both ESBL and Biofilm were exhibiting more resistance against common classes of antibiotics than the isolates producing ESBL alone, which proves that there is a significant correlation between ESBL production and biofilm production by Non fermentors. It is necessary to study each non-fermenting gram negative bacilli, especially isolated from hospital patients, in detail about their resistance pattern as well as other virulence factors. So also biofilm producing isolates especially from ICU patients require more attention in the selection of antibiotics.

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