Int.J.Curr.Microbiol.App.Sci (2018) *7*(1): 574-586

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 01 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.701.070

Study of Biofilm Production and Antimicrobial Susceptibility Pattern in Clinical Isolates of *Proteus* Species at a Tertiary Care Hospital

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Keywords

Proteus species, Biofilm, Antibiotic susceptibility

Article Info

Accepted:
06 December 2017
Available Online:
10 January 2018

A B S T R A C T

Proteus species are among the commonly implicated pathogens in hospital as well as community acquired infections. They cause nosocomial sinusitis, intra-abdominal abscesses, biliary tract infection, surgical site infection, soft tissue infection and osteomyelitis. They cause significant clinical infections, which are difficult to eradicate especially from hosts with wounds, catheterization, underlying diseases and in immunocompromised states. The therapeutic obstacles during *Proteus species* treatment can be connected with its ability to form biofilm, antibiotic resistance due to biofilm formation leading to persistence of infections. Hence the study is undertaken to find out the ability of Proteus species isolated from different clinical samples to form biofilm and their antibiotic susceptibility pattern. Total of 150 *Proteus* species isolates from different clinical samples such as urine, purulent material from wounds or abscesses, ear swabs, sputum, blood or aspirates (of joint fluid, pleural fluid, ascitic fluid and pus) collected from patients suspected of bacterial infection at Victoria Hospital, Vani Vilas Hospital, attached to Bangalore Medical College and Research Institute during the period November 2012-November 2014. Samples were collected after taking informed consent from all patients and were processed. The identification of *Proteus* species was done using standard biochemical tests. *Proteus* species thus identified were screened for biofilm production by tissue culture Plate (TCP) method, tube adherence method and Congo red agar method and studied for the antibiotic susceptibility patterns by in-vitro testing by Kirby-Bauer disc diffusion method performed according to CLSI recommendations. Out of the 150 *Proteus* species isolated from various clinical samples, 79 were from male and 71were from females. Majority of clinical samples from which Proteus species were isolated were from pus samples (91.33%). Among them, majority were from patients of thermal burns (34 %). *Proteus mirabilis* was the most isolated species (57.3%). For detection of biofilm, tissue culture plate method was considered as gold standard. Among the isolates, 48% were biofilm producers and 52% were non/weak biofilm producers. Among Proteus mirabilis, 52.32% were biofilm producers whereas among Proteus vulgaris, 42.59% were biofilm producers. Among the MDR isolates, 56.5% of the isolates showed biofilm production whereas 40(43.4%) of isolates were weak/non biofilm producers. Majority of *Proteus* isolates showed sensitivity to Imipenem (81.1%), Piperacillin + Tazobactum (83%). Sensitivity was highest with Congo red agar method and tube adherence methods in combination (86.11%). Congo red agar method showed better specificity 82.05% than other methods. There is an increase in isolation of *Proteus* species from wounds than from urinary tract infections with significant numbers showing biofilm forming capability. Tissue culture plate method was considered as gold standard method for biofilm detection in the present study. Also biofilm forming Proteus species isolates showed more antimicrobial drug resistance. Multi drug resistant Proteus isolates showed more biofilm forming ability indicating need for further studies on *Proteus* species and their ability to form biofilms. Also further studies on better understanding of bacterial biofilm interactions at molecular level for this species are necessary as they are more frequently being implicated in chronic infections and treatment failures. Also Tube adherence method and Congo red agar method in combination proved very effective for screening biofilm forming isolates. Though PCR technique is the most accurate and specific method, it is not readily available in resource limited settings and the need of the hour is rapid, cheap, cost effective and easily available methods for detecting biofilm formation. In view of emerging drug resistance and multidrug resistance exhibited by *Proteus species*, periodic review and formulation of antibiotic policy are needed. Indiscriminate use of antibiotic must be discouraged and therapy should be advocated as for as possible after the culture and sensitivity reports are available.

Introduction

Proteus species are among the commonly implicated pathogens in hospital as well as community acquired infections. (Mandell Gerald) The *Proteus* group belongs to the family of Enterobacteriaceae, the genus *Proteus*. *Proteus* species cause 5% of cases of hospital-acquired (UTI) and are responsible for 10–15% of cases of complicated UTI, primarily those associated with catheterization (Longo Dan *et al.,* 2011). *P. mirabilis* is the third most commonly isolated pathogen (after *Escherichia coli* and *Klebsiella pneumoniae*) of urinary tract infections. Among UTI isolates from chronically catheterized patients, the prevalence of *Proteus* is 20–45% (Longo Dan *et al.*, 2011). They also cause nosocomial sinusitis, intra-abdominal abscesses, biliary tract infection, surgical site infection, soft tissue infection and osteomyelitis.

The therapeutic obstacles during *Proteus* species treatment can be connected with its ability to form biofilm (Joanna Kwiecińska-Piróg *et al.,* 2013). Biofilms are bacterial populations that are enclosed in a matrix of extracellular polymeric substances (Mandell Gerald; Zubair *et al.*, 2011; Stoodley *et al.,* 2004). Biofilm forms on living (*e.g.* wounds) or non-living surfaces such as intravenous catheters, artificial joints, cardiac pacemakers and establishes a protective environment in natural, industrial and hospital settings. Biofilm-living bacteria are phenotypically different from their planktonic counterparts.

Biofilm forming cells exhibit antibiotic resistance to which their planktonic counterparts appear susceptible by in vitro testing. This is due to following reasons:

Nearness of cells within biofilm enhances the transfer of plasmids coding virulence factors and the mechanisms of antibiotic resistance.
Mucus and glycocalyx reduce antibiotic distribution into the deeper layers of the biofilm.

Quorum sensing (QS) enables transfer of information connected with biocidal agents' resistance and the mechanisms of their activation. Thus, biofilm forming bacteria are the cause of many chronic, recurrent and persistent infections (Stoodley *et al.*, 2004).

Concept of bacterial biofilms has emerged as a potential way to better understand how bacteria deter healing. Also according to a public announcement from National Institute of Health (NIH), more than 60% of all infections are caused by biofilm forming bacteria (Zubair *et al.,* 2011). Therefore, a better understanding of bacterial biofilms is needed, and this may ultimately result in development of novel therapeutics for the prevention and treatment. Hence the study is undertaken to find out the biofilm producers among *Proteus* species with their antibiotic susceptibility pattern.

Aims and objectives of the study

To isolate and identify *Proteus* species from various clinical specimens.

To determine the antibiotic susceptibility pattern of the isolates.

To determine biofilm producers among the isolates by tissue culture plate (TCP), Congo red agar and tube adherence methods and to compare these methods.

Materials and Methods

Source of data

This study was conducted from November 2012-November 2014. Different clinical samples such as urine, purulent material from wounds or abscesses, ear swabs, sputum,

blood or aspirates (of joint fluid, pleural fluid, ascitic fluid and pus) collected from patients suspected of bacterial infection were processed by microscopy and culture.

A total of 150 isolates of *Proteus* species isolated from such cases were identified to species level and studied for their ability to form biofilm by three phenotypic methods and their antimicrobial susceptibility patterns were studied.

Method of collection

The study population included both out patients and in patients attending different clinical departments of Victoria and Vani Vilas hospitals, Bangalore Medical College and Research Institute (BMC&RI). The specimens were transported to the laboratory within 2 hours and processed.

Inclusion criteria

Proteus species isolates from different clinical samples such as urine, purulent material from wounds or abscesses, ear swabs, sputum, blood or aspirates (of joint fluid, pleural fluid, ascitic fluid and pus) collected from patients suspected of bacterial infection.

Exclusion criteria

Any bacterial growth other than *Proteus* species.

Any fungal growth

Laboratory procedures

Clinical samples of pus, urine, sputum, blood and other samples sent to Microbiology laboratory, BMC & RI were processed. The specimens were brought to the laboratory within 2hrs of collection for processing.

Gram-stain

Smears were made from all samples except blood, heat-fixed and stained by Gram-stain. Smears were examined for the presence of pus cells and gram-negative organisms.

Culture

Pus, sputum and other samples were inoculated onto MacConkey agar and Blood agar. The media were incubated aerobically overnight at 37°C and observed for growth on the next day. Blood samples were inoculated in blood culture bottles were incubated at 37° C under aerobic conditions in the incubator for 7 days. The first subculture was done after 24 hours of incubation, the second on the third day and a final on the seventh day. On the next day it was inoculated onto MacConkey agar and Blood agar.

Urine sample was inoculated with standard loop on MacConkey agar [MA] and blood agar [BA]. After 24hrs of incubation at 37°C, cultures were further processed. Cultures yielding no growth and insignificant/mixed growth were excluded.

Depending on the morphology of colonies, the presumptive identification of the organism was made (Collee *et al.,* 1996):

Proteus species: On MA – Pale/ colorless colonies [non-lactose fermenters].

On BA – Colonies with characteristic swarming and fishy odour.

The isolates were bio-chemically identified by the following tests:

Proteus species: Oxidase non producer, Catalase producer, Methyl Red Positive, Vogues Proskauer variable, TSI agar showing K/A with gas and with abundant H_2S, Urease

produced, Citrate variable utilization, phenyl alanine deaminase producer.

Proteus mirabilis: Indole non producer Ampicillin sensitive (≥17 mm), Ampicillin resistant (≤13mm) and maltose non fermenter.

Proteus penneri: Indole non producer, Ampicillin resistant (≤13mm) and maltose fermenter.

Proteus vulgaris: Indole producer, salicin fermenter

Proteus hauseri: Indole producer, salicin non fermenter

Ampicillin: (10µg disc, Kirby Bauer disc diffusion test as per CLSI guidelines)

Antimicrobial susceptibility testing (Clinical and Laboratory Standard Institute, 2012)

Antimicrobial susceptibility testing of isolates will be done according to Kirby-Bauer disc diffusion method on Mueller Hinton Agar (MHA) for Amikacin (30µg), Gentamicin (10µg), Ampicillin (10µg), Aztreonam (30µg), Cefotaxime (30µg), Cotrimoxazole (Trimethoprim- sulfamethoxazole-1.25/23.75µg), Norfloxacin (10µg) (for urine isolates) Ceftriaxone (30µg), Imipenem (10µg), Piperacillin/tazobactam (100/10µg). Interpretation will be done according to CLSI recommendations.

Biofilm assay - Tissue Culture Plate (TCP) method

The biofilm assay described by Zubair *et al.,* (2011) is the first method adopted. Prior to biofilm production assay, the strains are cultured on Blood Agar and incubated overnight aerobically at 37°C. After verifying purity of the tested strain, several colonies with identical morphology are suspended in sterile physiological saline. The turbidity of the bacterial suspension is adjusted to 0.5 of the McFarland standard (~1.5 x 10^8 CFU/ml). Then 10 ml of trypticase soy broth (TSB) with 1% glucose is inoculated with a loopful of test organism from overnight culture on nutrient agar. The TSB broth is incubated at 37^{o}C for 24 hours. The culture is further diluted 1:100 with fresh medium and flat bottom tissue culture plates (96 wells) are filled with 200µl of diluted cultures individually. Uninoculated sterile broth served as blank. Positive and negative controls were also inoculated. The culture plates are incubated at 37ºC for 24 hours. After incubation, gentle tapping of the plates is done. The wells are washed with 200 µl of phosphate buffer saline (pH 7.2) four times to remove free-floating bacteria. Biofilms which remain adherent to the walls and the bottoms of the wells are fixed with absolute methanol and stained with 0.1% crystal violet. Excess stain is washed with deionized water and plates are dried properly. Optical densities (OD) of stained adherent biofilm are obtained with a micro ELISA auto-reader at wavelength of 570 nm. Experiments were performed in triplicate. For all tested strains and negative controls, the interpretation of biofilm production was done according to the criteria of (Stepanovic *et al.,* 2017).

The average OD values are calculated (from the inoculated triplets). The cut-off value (ODc) was established; the ODc is defined as three standard deviations (SD) above the mean OD of the negative control. The OD value of the tested strain is expressed as average OD value of the strain reduced by ODc value. ODc value should be calculated for each microtiter plate separately.

$ODc = Ø\ OD_{negative\ control} + 3 \times SD_{negative\ control}$

$OD = Ø\ OD_{tested\ strain} - OD_c$

Ø=Average, SD=Standard devaition

For easier interpretation of the results, strains may be divided into the following categories: strain not producing biofilm, strain weakly producing biofilm, strain moderately producing biofilm and strain strongly producing biofilm. This categorization should be based of the previously calculated OD values (for this type of data interpretation the OD value of the strain should not be reduced by OD_c value).

$OD \leq OD_c$ →Strain not producing biofilm

$OD_c < OD \leq 2x\ OD_c$ →Strain weakly producing biofilm

$2x\ OD_c < OD \leq 4x\ OD_c$ →Strain moderately producing biofilm

$4x\ OD_c < OD$ →Strain strongly producing biofilm

Biofilm production by using Congo Red Agar (CRA) medium

It is the second method done as described by Freeman *et al.,* (1989) and will be the second method adopted. It is a qualitative method to detect biofilm production by using Congo Red Agar (CRA) medium. CRA medium is prepared with brain heart infusion broth 37 g/L, sucrose 50 g/L, agar 10 g/L and Congo Red indicator –0.8 g/L. First Congo red stain is prepared as a concentrated aqueous solution and autoclaved (121ºC for 15 minutes) separately from the other medium constituents. Then it is added to the autoclaved brain heart infusion agar with sucrose at 55ºC. CRA plates will be inoculated with test organisms and incubated at 37ºC for 24 to 48 h aerobically. Black colonies with a dry crystalline consistency indicate biofilm production.

Biofilm production by tube adherence method

It is third method done as described by (Christensen *et al.,* 1982) Suspension of tested strains will be incubated in the glass tubes containing Brain Heart Infusion Broth aerobically at the temperature of 35^{o}C for a period of two days.

Then the supernatant discarded and the glass tube will be 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 will be considered negative.

The amount of biofilm formed was scored as 1-weak/none, 2-moderate and 3-high/strong. (Hassan Afreenish *et al.,* 2011).

Fig.1 *Proteus* species showing non lactose fermenting colonies on MacConkey agar

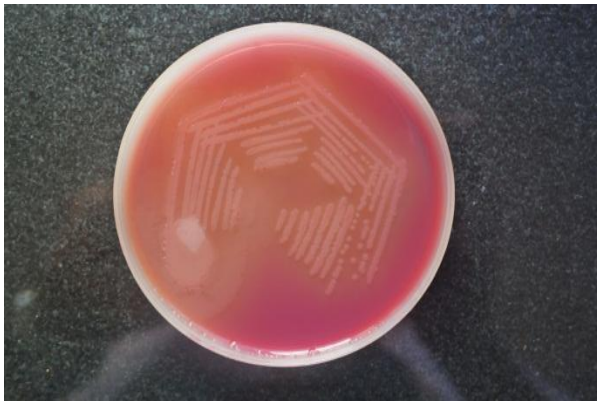

Fig.2 *Proteus* species negative (left) and positive (right) biofilm producer by Congo red agar method

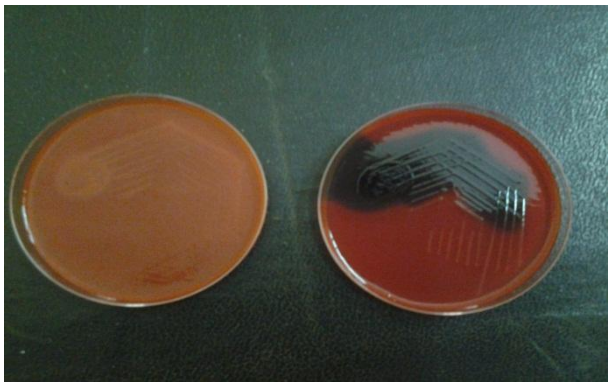

Fig.3 Tube adherence method: left showing 0 (non-biofilm producer), 1 (mild biofilm producer), 2 (moderate biofilm producer), 3(strong biofilm producer) from top to bottom respectively. Right set of tubes are test strains

Fig.4 Tissue culture plate method: 1: Strong biofilm producer. 2: Moderate biofilm producer 3: Mild biofilm producer 4: non biofilm producer

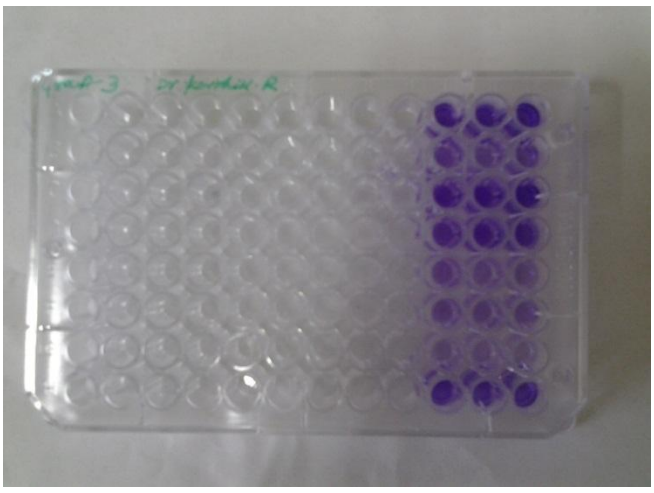

Fig.5 Antibiotic susceptibility pattern of *Proteus species* isolates

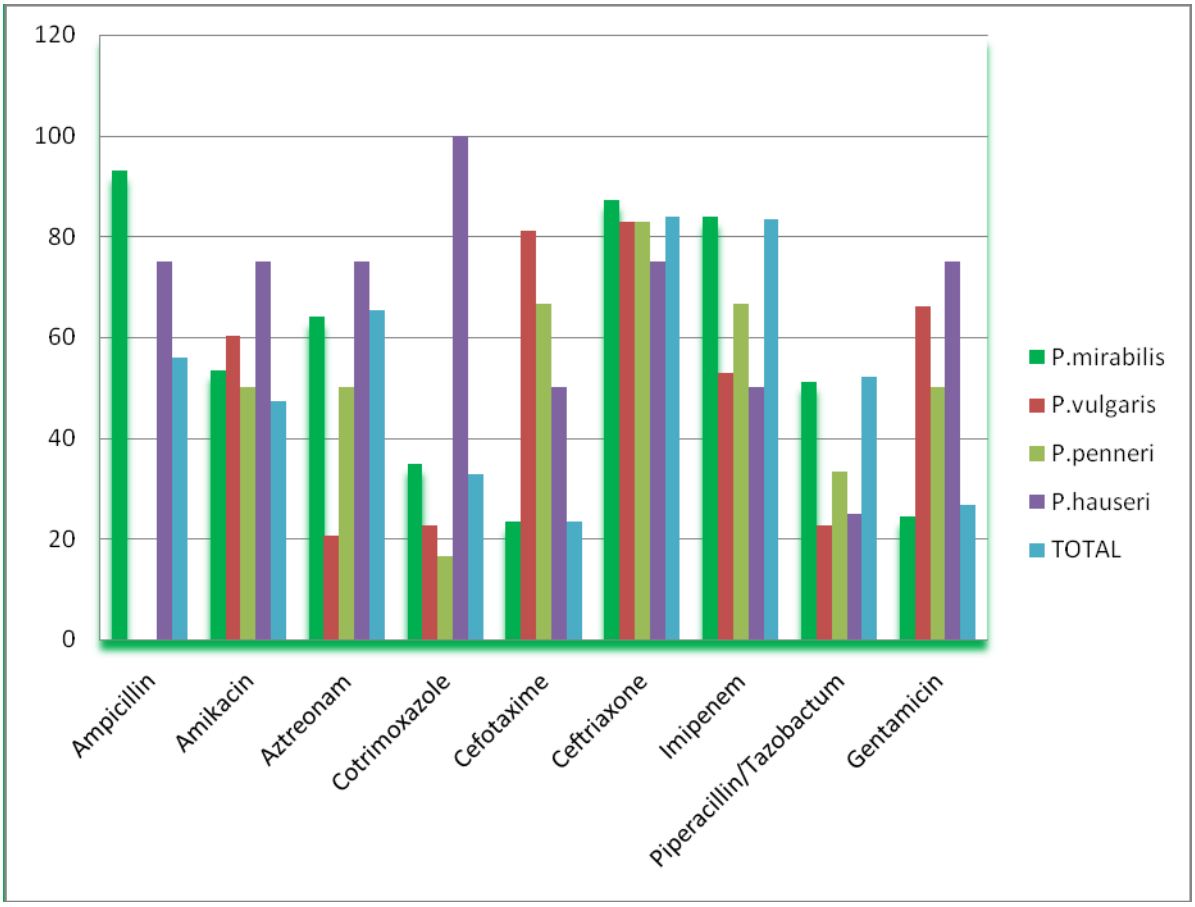

Fig.6 Antimicrobial resistance pattern among biofilm producers and non-producers

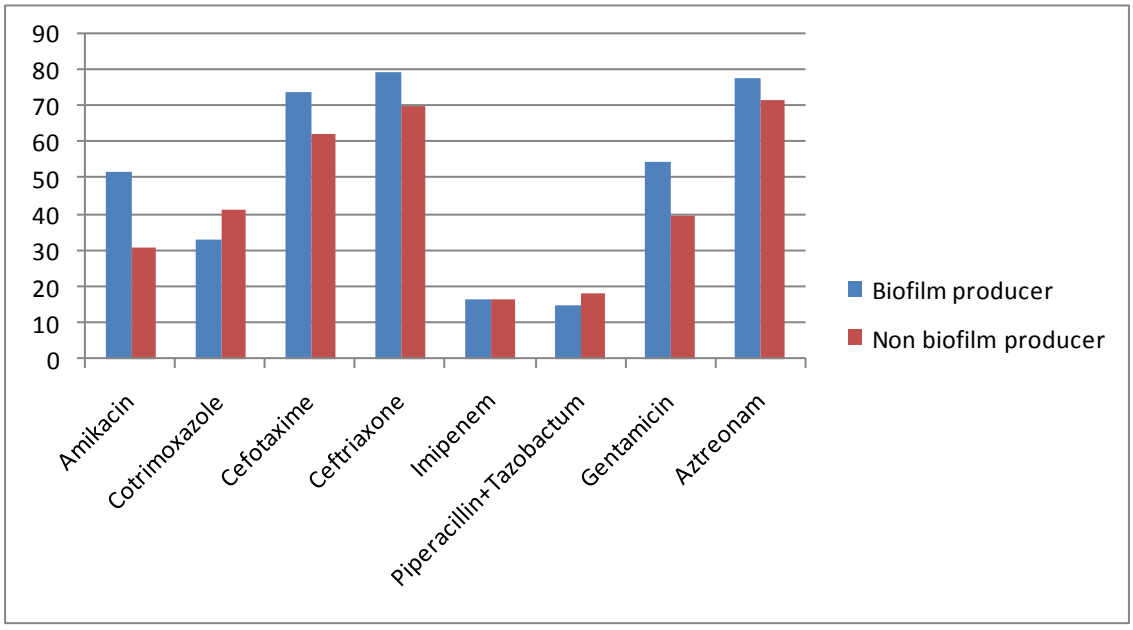

Table.1 Correlation between tube adherence method and tissue culture plate method (Gold standard-Mathur *et al.,* 2006)

	Positive	**Negative**	**Total**	
Correlating	56	60	121	Positive predictive value: 81.1%
Not correlating	16	18	29	Negative predictive value:80.2%
Total	72	78	150	
	True positive rate: (Sensitivity):77.7%	False positive rate: 23%	Accuracy: 80.66%	
	False negative rate: 22.2%	True negative rate: 76.9%		

Table.2 Correlation between Congo red agar method and tissue culture plate method (Gold standard-Mathur *et al.,* 2006)

	Positive	**Negative**	**Total**	
Correlating	30	64	94	Positive predictive value: 68.18%
Not correlating	42	14	56	Negative predictive value:60.3%
Total	72	78	150	
	True positive rate: (Sensitivity):41.6%	False positive rate: 17.9%	Accuracy: 64%	
	False negative rate: 58.3%	True negative rate (Specificity): 82.05%		

Table.3 Correlation between Congo red agar method +tube adherence and tissue culture plate method (Gold standard-Mathur *et al.,* 2006)

	Positive	**Negative**	**Total**	
Correlating	62	36	98	Positive predictive value: 59.6%
Not correlating	10	42	52	Negative predictive value: 78.2%
Total	72	78	150	
	True positive rate: (Sensitivity): 86.11%	False positive rate: 53.8%	Accuracy: 65.3%	
	False negative rate: 13.8%	True negative rate (Specificity): 46.1%		

Quality control

The following international reference strains will be used as controls: Non-biofilm producers *Staphylococcus epidermidis* ATCC 12228 (negative control). For positive control, *Proteus* mirabilis strain from study which produced positive result by all three methods was used after cross checking with department of microbiology, ESIC PGIMSR model hospital Rajajinagar, was used.

Considering Tissue culture method as gold standard, (Mathur *et al.,* 2006), tube adherence method showed 80.66% correlation. Sensitivity was 77.7% and specificity was 76.9%.

Tube adherence showed good correlation (accuracy) with TCP method (80.66%) which was better than congo red agar method (64%) and Congo red and tube adherence method combined.

Sensitivity was highest with Congo red agar method and tube adherence methods combined (86.11%).

Tube adherence method showed good sensitivity (77.7%) when compared with Congo red agar method which showed lowest sensitivity (41.6%).

Congo red agar method showed better specificity 82.05% compared to tube adherence method (76.9%) and CRA and TA combined (46.1%).

Tube adherence method had better positive and negative predictive value (81.1 and 80.2% respectively) compared to other methods (Table 1–3).

Biofilm producer showed more resistance to Amikacin, Cefotaxime, Ceftriaxone, Gentamicin and Aztreonam in comparison to non-biofilm producing isolates. *Proteus* species are widespread in the environment and are part of the normal flora of the human gastro intestinal tract. *Proteus* species currently rank third as cause of uncomplicated cystitis, pyelonephritis, and prostatitis particularly when they are hospital acquired.

They have also been implicated in bacteraemia, empyema, osteomyelitis, burn wound infections. Among the various virulence factors of *Proteus* species, their ability to form biofilm is very important due to the fact that it leads to antimicrobial resistance and treatment failures, chronic infections, embolism and distant site infections. The treatment of *Proteus* species infections is increasingly becoming difficult because of their ability to form biofilms and multidrug resistance exhibited by these organisms. The results of 150 *Proteus* species isolates from different clinical cases were studied compared and discussed with the results of other studies.

In the present study majority of the *Proteus* species isolates were between the age group 20-59 years which correlates with (Feglo *et al.,* 2010) wherein maximum number of *Proteus* species isolated were between 20-59 years of age. In the present study there is male preponderance. This finding co related with (Saleh *et al.,* 2013). Males were found to be more vulnerable than females in acquiring *Proteus* species infections. From comparing with other studies it was observed that the present study clearly showed predominance of pus samples from where *Proteus* species were isolated followed by urine and endotracheal aspirates. (Nita pal *et al.,* 2014) showed similar pattern of isolation of *Proteus* species in clinical specimens. *Proteus* seems to be a common cause of wound infections in India and other third world countries in comparison to those from Europe where they cause more urine infections than wound infections.

Comparison of biofilm production among *Proteus* species isolates by tissue culture plate method

The therapeutic obstacles during *P. mirabilis* treatment can be connected with its ability to form biofilm. Biofilm is a formation of communicating microorganisms, adhering to certain surfaces and to neighbouring cells, covered with an extracellular matrix. It may consist of one or various species. The ability to form biofilm promotes the development and chronicity of infections. Biofilm can form on non-living as well as living surfaces such as wound surface. Also biofilm organism show increased antimicrobial resistance. Among the *Proteus* species isolates the present study showed 48% as biofilm producers and 52 % as biofilm non producers which correlated with other similar study done by Bardsiri Shikh-Bardsiri and Mohammad Reza Shakibaie (2013) who reported 43% of isolates as non/weak biofilm producers and 57% as biofilm producers. Among moderate biofilm producers, the present study (29.4%) correlated with Zubair *et al.,* (2011) (31%). In the present study tube adherence method showed 77.7% sensitivity and 76.9% specificity with accuracy of 80.66%. The sensitivity and accuracy correlated well study by Mathur *et al.*, (2006) which showed 73.6% sensitivity and 82.7% accuracy as well as Bose *et al.,* (2009) which showed 76.27% sensitivity and 81% accuracy. In the present study, Congo red agar method showed 41.6% sensitivity and 81.05% specificity with accuracy of 64%. The present study showed low sensitivity but higher specificity as shown by Mathur *et al.,* (2006) and Bose *et al.,* (2009) in their studies (6.8% and 8.25% sensitivity respectively and 90.2% and 96.34% specificity respectively). Accuracy of the present study (64%) was similar to Bose *et al.,* (2009) (55%). The findings of the present study indicates high level of multi drug resistant *Proteus* isolates with *Proteus penneri* being always multi drug resistant as similar results were found in other studies. The results of *Proteus mirabilis* (62.7%) and *Proteus vulgaris* (67.3%) correlated with Feglo *et al.,* (2010) 84.6% and 93.4% respectively. In the present study, *Proteus mirabilis* showed more resistance to commonly used antibiotics such as Ceftriaxone, Cefotaxime, Aztreonam. There is also increasing resistance to Gentamicin. High resistance to Cefotaxime (65.11%) was seen as reported by others (Donlan and Costerton, 2002). Amikacin resistance was seen in 46.5% of cases, similar to Jitendra Kumar Pandey *et al.,* (2013) (60%) and Nita Pal *et al.,* (2014) (65%). There was high resistance to Aztreonam (75.5%) which corroborates with earlier observations (Wenner and Rettger, 1919).

In the present study the most sensitive drug is Imipenem with 87.3%. This is correlated with Nita Pal *et al.,* (2014) with 94% sensitivity. The least sensitive drug is Aztreonam with 24.5% which corroborated with Jitendra Kumar Pandey *et al.,* (2013). In the present study, *Proteus vulgaris* showed more resistance to commonly used antibiotics such as Ceftriaxone, Cefotaxime, Aztreonam. There is also increasing resistance to Gentamicin. High resistance to Cefotaxime (79.24%) was seen which correlated with Nita Pal *et al.,* (2014) Amikacin resistance was seen in 33.9% of cases, similar to Jitendra Kumar Pandey *et al.,* (2013) (35%). There was high resistance to Aztreonam (77.3%) which corroborates with Jitendra Kumar Pandey *et al.,* (2013). In the present study the most sensitive drug is Imipenem with 81.14%.This is correlated with Jitendra Kumar Pandey *et al.,* (2013) with 80% sensitivity. The least sensitive drug was Cefotaxime (79.24% resistance) which correlated with Nita Pal *et al.,* (2014). In the present study, *Proteus penneri* showed more resistance to commonly used antibiotics such

as ceftriaxone, Aztreonam. High resistance to ceftriaxone (83.4%) and Aztreonam (66.67%) was seen which correlated with Jitendra Kumar Pandey *et al.,* (2013). Amikacin resistance was seen in 50% of cases, similar to Jitendra Kumar Pandey *et al.,* (2013) (60%).

Proteus species are important cause of nosocomial infections including uncomplicated cystitis, pyelonephritis, and prostatitis. They have also been implicated in bacteraemia, empyema, osteomyelitis, burn wound infections. They are associated with prolonged hospitalization andthe complications of long-term urinary catheterization. Also their ability to form biofilm is implicated in chronic infections and increased drug resistance. In the present study 150 *Proteus* species isolates from different clinical conditions were studied for their identification, antibiotic susceptibility pattern and their ability to form biofilm in vitro by three phenotypic methods which included tissue culture plate method, tube adherence method and Congo red agar method.

The majority of *Proteus* species isolates were between the age group 20-59 yrs. Among the 150 isolates 52.66% isolates were from male and 47.3 % from females. Among the 150 isolates, 57.3% were *Proteus mirabilis*, followed by *Proteus vulgaris* 36%, *Proteus penneri* 4% and *Proteus hauseri* 2.7%. Among the total isolates, 48.66% were biofilm producers and remaining were non biofilm producers. Among the biofilm producers, 38.8% were strong and 61% were moderate biofilm producers. Also tube adherence method for biofilm detection showed good correlation with tissue culture plate method and had good sensitivity, specificity and accuracy in comparison to Congo red agar method in biofilm detection. Congo red agar method and tube adherence method in combination showed higher sensitivity in biofilm detection compared to those methods used alone. AST of the isolates showed maximum sensitivity to Imipenem and Piperacillin/tazobactum followed by Cotrimoxazole, Amikacin, Gentamicin, Cefotaxime and least for Ceftriaxone and Aztreonam. Also majority of the isolates were multi drug resistant (MDR) and these MDR isolates showed better biofilm forming capacity than non MDR isolates. Also biofilm producers were more resistant to antimicrobial agents than non-biofilm producers.

The above results clearly points to the medical importance of biofilms. Tube adherence method and Congo red agar methods in combination can be used for screening isolates for biofilm forming ability since tissue culture plate method is more laborious and technically demanding procedure. Also there is an increase in isolation of *Proteus* species from wounds than from urinary tract infections with significant numbers showing biofilm forming capability and antimicrobial drug resistance indicating need for further studies on *Proteus* species. Tube adherence method and Congo red agar method in combination proved very effective for screening biofilm forming isolates. Though PCR technique is the most accurate and specific method for detecting the ability to form biofilm, it is not readily available in resource limited settings and the need of the hour is rapid, cheap, cost effective and easily available methods for detecting biofilm formation. However, further studies on better understanding of bacterial biofilm interactions at molecular level for this species are necessary as they are more frequently being implicated in chronic infections and treatment failures. In view of emerging drug resistance and multidrug resistance exhibited by *Proteus species*, periodic review and formulation of antibiotic policy are needed. Indiscriminate use of antibiotic must be discouraged and

therapy should be advocated as for as possible after the culture and sensitivity reports are available.

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How to cite this article:

Karthik, R., R. Ambica and Nagarathnamma, T. 2018. Study of Biofilm Production and Antimicrobial Susceptibility Pattern in Clinical Isolates of *Proteus* Species at a Tertiary Care Hospital. *Int.J.Curr.Microbiol.App.Sci.* 7(01): 574-586.
doi: https://doi.org/10.20546/ijcmas.2018.701.070