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Prevalence of *Proteus* species with reduced susceptibility to imipenem isolated from a tertiary referral hospital in Malaysia

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ABSTRACT

Aims: *Proteus* species are implicated as serious causes of various human infections. Imipenem has been used to treat infections caused by these organisms. However, *Proteus* spp. are known to have reduced susceptibility and have elevated minimum inhibitory concentration (MIC) towards imipenem. The aim of this study was to determine the prevalence of *Proteus* species with reduced susceptibility to imipenem and the antibiotic susceptibility pattern for each *Proteus* species.
Methodology and results: A total of 204 *Proteus* isolates were collected from routine samples. All isolates were identified by using VITEK® 2 GN ID card. Antibiotic susceptibility tests were done by using disc diffusion method and imipenem E-test. While 5.9% of the *Proteus* isolates showed reduced susceptibility towards imipenem by disc diffusion, only 1% (2 out of 204 isolates) has reduced susceptibility by E-test.
Conclusion, significance and impact of study: The prevalence of *Proteus* species with reduced susceptibility to imipenem is still low. The imipenem zone diameter does not truly reflect the MIC value and thus, in any isolates which are tested to have reduced susceptibility or resistant to imipenem should always be followed by imipenem MIC method.

Keywords: Proteus, imipenem, reduced susceptibility, resistant

INTRODUCTION

The genus *Proteus* is grouped together with *Providentia* and *Morganella* genera under the Proteeae tribe. They are related members of the Enterobacteriaceae families of Gram-negative bacilli that are motile, non-lactose fermenter and produce phenylalanine deaminase (Donnenberg, 2011). The genus *Proteus* currently consists of five named species (*P. mirabilis*, *P. penneri*, *P. vulgaris*, *P. myxofaciens*, and *P. hauseri*) and three unnamed genomospecies (*Proteus* genomospecies 4, 5, and 6) (O'Hara *et al.*, 2000). Nevertheless, *P. mirabilis* and *P. vulgaris* account for the vast majority of clinical isolates in this genus.

Proteus species contribute to significant implication in the hospital setting. This pathogen is known to cause bloodstream infection, opportunistic infection, wound infection and one of the main causes of urinary tract infections (UTIs) among hospitalised patients with indwelling urinary catheters and urinary tract calculi.

Imipenem was reported to be the most effective antibiotic against *Proteus* species (Bahashwan and El Shafey, 2013). Based on the Clinical and Laboratory Standards Institute (CLSI) guideline, it is known that *Proteus* species tends to have higher imipenem MIC (e.g. MICs in the intermediate or resistant range) compared to other enterobacteriaceae. A study in Europe had shown that, 7% of *P. mirabilis* isolates has reduced susceptibility towards imipenem compared to less than 1% for meropenem (Mutnick *et al.*, 2002). Another study had found isolates (all 16 isolates) which were fully or intermediately susceptible to imipenem (MICs of 2 to 8 mg/L) and meropenem (MICs of 0.25 to 2 mg/L) by broth microdilution (Miriagou *et al.*, 2010) . Neuwirth *et al*. in his study in 1995, revealed findings suggesting that imipenem resistance in *P. mirabilis* might result from altered penicillin-binding proteins (PBPs) even though the strain is susceptible to other beta-lactams.

To our knowledge, there is no published data from Malaysia specifically addressing the prevalence of *Proteus* species with reduced susceptibility towards imipenem.

Thus, the aim of our study was mainly to determine the prevalence of *Proteus* species with reduced susceptibility to imipenem. Furthermore, we would like to determine the antibiotic susceptibility pattern for each *Proteus* species and to compare the imipenem

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susceptibility using disc diffusion size and E-test method among the *Proteus* species isolates.

MATERIALS AND METHODS

Specimen collection

A total of 204 *Proteus* spp. were included in the study. Non-repeated strains of *Proteus* species isolated from various clinical specimens in the Microbiology Laboratory, Hospital Kuala Lumpur from June 2015 till June 2016 were evaluated. The specimens included were blood, tissue, bone, urine, pus, vaginal swabs, respiratory samples, cerebrospinal fluid and sterile body fluids. The *Proteus* spp. isolated were subcultured on nutrient agar slants and froze at ─20 °C until further testing. The type of infections, specimens, *Proteus* species isolated and the antibiotic susceptibility pattern were recorded.

Cultivation and identification

All specimens were aseptically inoculated on blood agar and Mac Conkey agar. Cystine-Lactose-Electrolyte-Deficient (CLED) agar was added for urine specimens. The characteristic swarming colonies on blood agar and oxidase-negative non-lactose fermenting organism on Mac Conkey agar were identified. Presumptive *Proteus* colonies were identified through extended biochemical tests; triple sugar iron, indole, methyl red, citrate, motility, ornithine, phenylalanine deaminase, oxidative-fermentative test, urea test and malonate. VITEK® 2 GN ID card (bioMérieux, Marcy-l'Etoile, France) was used for further confirmation of identification.

Antimicrobial susceptibility test

Mueller-Hinton agar was prepared from a commercially available dehydrated base according to the manufacturer's instructions. The agar was inoculated with *Proteus* species using the direct colony suspension method. The inoculum was prepared by making a direct colony suspension from an 18 to 24-hour culture. The suspension was adjusted to match the 0.5 McFarland turbidity standards, using saline and a vortex mixer.

Disc diffusion method

The Kirby-Bauer method was used as recommended by the CLSI guideline. The predetermined battery of antimicrobial discs were applied and lightly pressed onto the surface of the inoculated agar plate. The plates were then inverted and incubated at 35 °C for 16 to 18 h. The antibiotics tested include; ampicillin (10 µg), cefotaxime (30 µg), cefepime (30 µg), ceftazidime (30 µg), cefoxitin (30 µg), amoxicillin-clavulanate (20/10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), ertapenem (10 µg), meropenem (10 µg), imipenem (10 µg), cefoperazone (75 µg), cefuroxime (30 µg), gentamicin (10 µg), amikacin (30 µg), ampicillin-sulbactam (10/10 µg), piperacillin-tazobactam (100/10 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg) (for non-urine specimens), nitrofurantoin (300 µg) (for urine specimens) and polymyxin B (300 U). Interpretative criteria of antibiotic susceptibility were based on the 2015 CLSI breakpoints.

Table 1: Proportion of *Proteus* species identified using Vitek®2 GN.

Proteus species identified (n=204)	n (%)
P. mirabilis	194 (95.1)
P. penneri	7 (3.4)
P. hauseri	2 (1.0)
P. vulgaris	1 (0.5)

Table 2: Distribution of *Proteus* sp. according to the type of specimen.

Type of specimen	n (%)
Urine	64 (31.4)
Pus	52 (25.5)
Tissue	44 (21.6)
Blood	16 (7.8)
Bone	15 (7.4)
Respiratory	9 (4.4)
Peritoneal fluid	2 (1.0)
Aspirate (from blister)	1 (0.5)
Cerebrospinal fluid	1 (0.5)
Total	204 (100)

E-test method

E-test is a quantitative method for antimicrobial susceptibility testing which applies both the dilution of antibiotic and diffusion of antibiotic into the medium. In the present study, all *Proteus* isolates were tested with Imipenem E-test. Result interpretation was based on 2015 CLSI breakpoints (Sensitive, ≤1 mcg/mL; Intermediate, 2 mcg/mL; Resistant, ≥4 mcg/mL).

RESULTS

Proteus species isolated

A total of 204 *Proteus* species were included in this study. *Proteus mirabilis* is the most common *Proteus* species isolated (95.1%) followed by *P. penneri* (3.4 %), *P. hauseri* (1.0%) and *P. vulgaris* (0.5%) (Table 1).

Most of the *Proteus* sp. was isolated from urine specimen which accounted for 31.4% (n=64) followed by pus (25.5%), tissue (21.6%), blood (7.8%), bone (7.4%), respiratory (4.4%) and peritoneal fluid (1.0%). *Proteus* sp.

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Table 3: Distribution of *Proteus* sp. according to the type of infection.

Type of infection	n (%)
Urinary tract infection	64 (31.4)
Skin & soft tissue infection (SSTI)	61 (29.9)
Diabetic foot infection	43 (21.1)
Bloodstream infection	16 (7.8)
Respiratory tract infection	9 (4.4)
Septic arthritis	4 (2.0)
Osteomyelitis	3 (1.5)
Ear infection	2 (1.0)
Others	2 (1.0)
Total	204 (100)

Table 4: Susceptibility of *Proteus penneri*, *Proteus hauseri* and *Proteus vulgaris* towards different antibiotics.

Antibiotics	*P. penneri* (n=7)	*P. hauseri* (n=2)	*P. vulgaris* (n=1)
Ampicillin	0	100	0
Cefotaxime	85.7	100	100
Cefepime	100	100	100
Ceftazidime	100	100	100
Cefoxitin	85.7	100	100
Amoxycillin-clavulanate	85.7	100	100
Trimethoprim-sulfamethoxazole	71.4	100	100
Ertapenem	100	100	100
Meropenem	100	100	100
Imipenem	85.7	100	100
Cefoperazone	14.3	100	100
Cefuroxime	0	100	0
Gentamicin	85.7	100	100
Amikacin	100	100	100
Ampicillin-sulbactam	85.7	100	100
Piperacillin-tazobactam	100	100	100
Ciprofloxacin	100	100	100
Chloramphenicol	33.3	100	100
Polymyxin B	0	0	0

were also isolated from blister aspirate and cerebrospinal fluid (0.5% each) (Table 2). The greatest number of *Proteus* sp. were isolated from patients with urinary tract infection (UTI) (31.4%), followed by skin and soft tissue infection (SSTI) (29.9%), diabetic foot infection (21.1%), bloodstream infection (7.8%), and respiratory infection (4.4%). *Proteus* sp. were also isolated from cases of septic arthritis, osteomyelitis and ear infection which accounted 2.0%, 1.5% and 1.0% respectively. Other infections that isolated *Proteus* sp. are cases of meningitis and peritonitis (1.0% each) (Table 3).

Antibiotic susceptibility pattern of *Proteus* species

The antibiotic susceptibility testing was carried out on all *Proteus* sp. isolates (*P. mirabilis*, *P. penneri*, *P. vulgaris*, and *P. hauseri*). Chloramphenicol discs were added to non-urine specimens (n=140) while nitrofurantoin discs for urine samples (n=64). Polymyxin B was used as one of the identification of *Proteus* spp. as they are innately resistant to this antibiotic. All *Proteus* sp. isolated were susceptible to ertapenem and meropenem but 12 (5.9%) of them show reduced susceptibility towards imipenem. More than 90% of the *Proteus* sp. were susceptible to ceftazidime, cefoxitin, amikacin and piperacillin-tazobactam representing 92.6%, 94.6%, 97.1% and 99.0%; respectively. More than 50% of the isolates were resistant to ampicillin (58.8%) and trimethoprim-sulfamethoxazole (55.9%) (Figure 1).

Proteus mirabilis isolates were largely susceptible to cephalosporin group, beta lactam-beta lactamase inhibitor, ciprofloxacin and aminoglycosides antibiotics. However, isolates from urine specimens were resistant to nitrofurantoin. Other antibiotics demonstrated variable susceptibility pattern. All *P. mirabilis* isolates were susceptible to ertapenem and meropenem. However, one *P. mirabilis* isolate was resistant to imipenem, 8 were intermediate while the remaining 186 (95.9%) were susceptible to imipenem (Figure 2). All *P. penneri* isolates (n=7) were resistant to ampicillin and cefuroxime. One *P. penneri* isolate was resistant to imipenem but susceptible to meropenem and ertapenem. Six isolates had reduced susceptibility to cefoperazone (Table 4). All *P. hauseri* (n=2) isolates were susceptible to all antibiotics tested. *Proteus vulgaris* (n=1) isolate was resistant only to ampicillin and cefuroxime.

Table 5 summarizes the overall susceptibility of *Proteus* sp. isolates (n=204) by imipenem disc and imipenem E-test. One hundred ninety two (94.1%) isolates were imipenem sensitive by disc and E-test. Intermediate susceptibility to imipenem was detected by disc in 8 (3.9%) isolates which became sensitive when tested by E-test. Four isolates (2.0%) were resistant by imipenem disc in which 2 isolates became susceptible, 1 became intermediately sensitive and another isolate was resistant when tested with imipenem E-test. A total of 204 isolates were tested with imipenem E-test (Table 6). Out of this, 202 (99%) isolates were susceptible. Only 2 *P. mirabilis* isolates had reduced susceptibility towards imipenem. One isolate had intermediate result with MIC: 2 mcg/mL and another 1 isolate was resistant with MIC: 32 mcg/mL to imipenem E-test.

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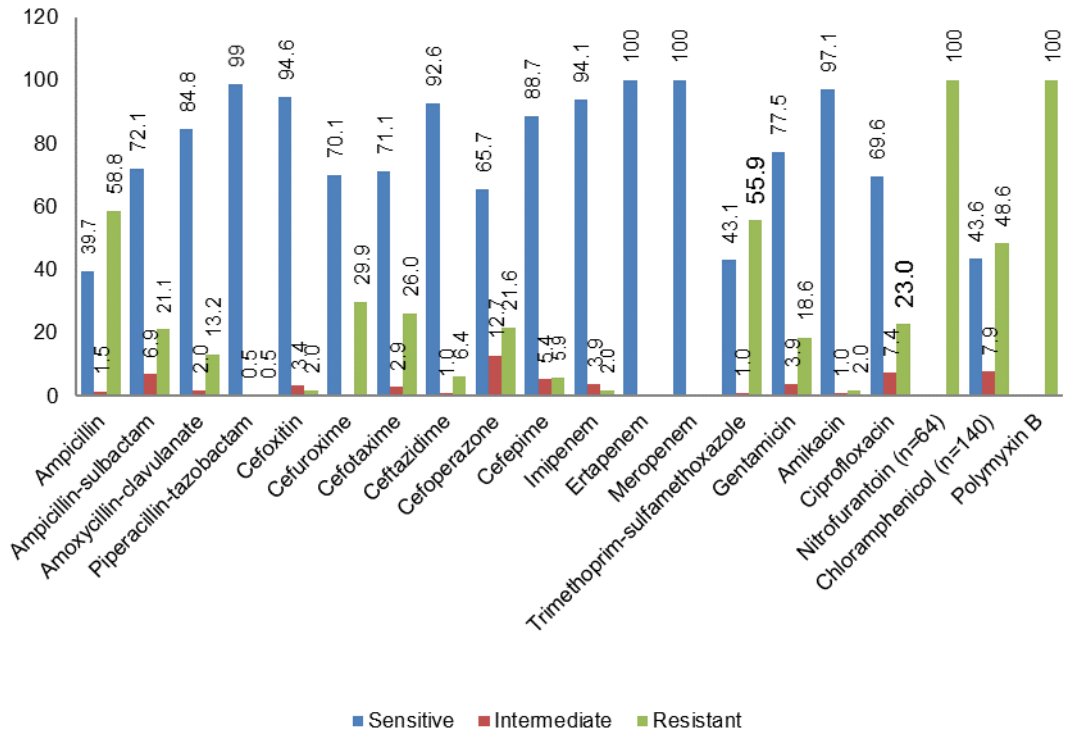

Figure 1: Percentage (%) of antimicrobial susceptibility pattern for all *Proteus* isolates (n=204) to different antibiotics.

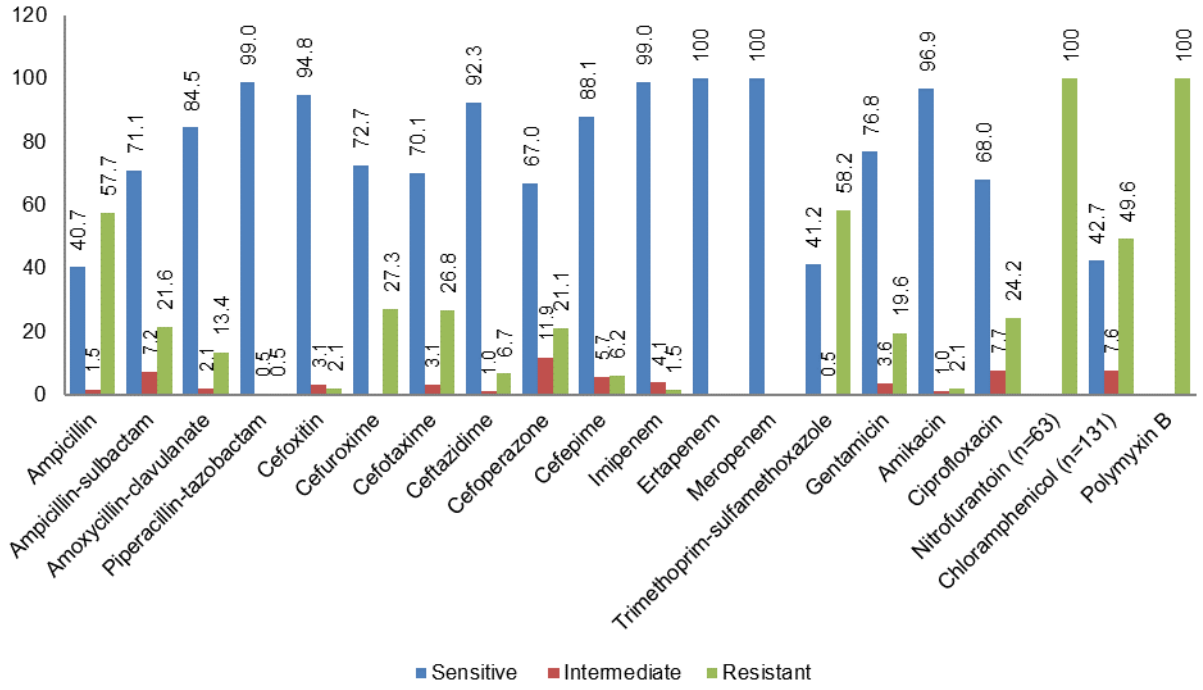

Figure 2: Percentage (%) of antimicrobial susceptibility pattern for *Proteus mirabilis* (n=194) to different antibiotics.

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Table 5: Imipenem disc susceptibility results and their MICs (minimum inhibitory concentration).

Imipenem disc	Imipenem MIC (E-test) susceptibility			Total
	Sensitive	Intermediate	Resistant	
Sensitive	192 (94.1%)	0	0	192 (94.1%)
Intermediate	8 (3.9%)	0	0	8 (3.9%)
Resistant	2 (1.0%)	1 (0.5%)	1 (0.5%)	4 (2.0%)
Total	202 (99.0%)	1 (0.5%)	1 (0.5%)	204 (100%)

Table 6: Distribution of imipenem E-test sensitivity according to each *Proteus* species.

Proteus species	Imipenem E-test sensitivity			Total
	Sensitive	Intermediate	Resistant	
P. mirabilis	192 (94.1%)	1 (0.5%)	1 (0.5%)	194 (95.1%)
P. penneri	7 (3.4%)	-	-	7 (3.4%)
P. vulgaris	1 (0.5%)	-	-	1 (0.5%)
P. hauseri	2 (1.0%)	-	-	2 (1.0%)
Total	202 (99.0%)	1 (0.5%)	1 (0.5%)	204 (100%)

DISCUSSION

Proteus species are isolated from various anatomical sites and have been implicated in several human infections. We had identified 4 *Proteus* species (*P. mirabilis*, *P. penneri*, *P. hauseri* and *P. vulgaris*) to be associated with infections from different clinical sites. Most literature reports that *P. mirabilis* is the most common *Proteus* sp. isolated from clinical specimens (Feglo *et al.*, 2010; Pal *et al.*, 2014). In this study, the most isolated species was *P. mirabilis* which accounted for 95.1% (194 out of 204) of samples.

Relating to the type of specimen associated with *Proteus* spp., urine is the highest type of specimen which accounted for 31.4%. This result is consistent with other studies done in Poland and Trinidad by Reslinski *et al.* (2005) and Orrett (1999) respectively who reported that *Proteus* species were more commonly encountered in urine than in other clinical specimens. On the other hand, studies by Newman *et al.* (2011) and Feglo *et al.* (2010) in Ghana and Yah *et al.* (2001) in Nigeria have described that *Proteus* spp. isolates were mostly recovered from wound swabs. Our results have also demonstrated *Proteus* spp. isolates were most frequently isolated from UTI. However there are limited past studies which documented the type of infection associated with *Proteus* spp.

The antimicrobial susceptibility patterns of *Proteus* isolates in this study were mostly represented by *P. mirabilis*. *Proteus* isolates are intrinsically resistant to polymyxin B and nitrofurantoin as shown in this study. All of the *Proteus* isolates were generally susceptible to the cephalosporin group (70.1-92.6%), beta lactam-beta lactamase inhibitor (72.1-99.0%), ciprofloxacin (69.6%) and aminoglycosides (76.8-96.9%). These findings were comparable with the study done by Bahashwan *et al.* (2013) except that his study had reported high levels of ciprofloxacin resistance among *P. mirabilis* isolates. On the other hand, more than 50% of the isolates recovered were resistant to ampicillin and trimethoprim-sulfamethoxazole while nearly half of the non-urine isolates were resistant to chloramphenicol. Feglo *et al.* (2010) had also found similar results for the respective antibiotics.

In the present study, all 204 isolates were susceptible towards ertapenem and meropenem while 5.5% showed reduced susceptibility to imipenem by disc diffusion. This finding is much lower compared to previous studies which gave rates of reduced susceptibility to imipenem between 7 to 32.8% (Mutnick *et al.*, 2002; Bahashwan *et al.*, 2013; Pal *et al.*, 2014; Wang *et al.*, 2014). The mechanism of resistance might result from altered PBPs (Neuwirth *et al.*, 1995) or due to imipenem selective pressure by means of modulated efflux pump and porin genes (Pavez *et al.*, 2016). Therefore, the susceptibility of *P. mirabilis* to carbapenems should be determined by ertapenem, meropenem, or doripenem (Wang *et al.*, 2014) by either disk diffusion or MIC method (CLSI, 2015).

We also did a comparison between the imipenem zone diameter and individual E-test value which showed that 12 *Proteus* isolates were either intermediately susceptible or resistant by imipenem disc diffusion while only 2 isolates had reduced susceptibility when tested using E-test. Our findings showed that the zone diameter does not truly reflect the MIC value and thus, in isolates which are tested to have reduced susceptibility or

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resistant to imipenem should always be followed by MIC testing (CDC, 2010). The E-test is easy to execute as the application is the same as the diffusion agar method. In comparison to broth dilution, E-test provides quantitative wide-range MICs, simple and the results are reproducible.

CONCLUSION

In conclusion, the prevalence of *Proteus* species with reduced susceptibility to imipenem is still low (1%). However, all isolates had shown susceptibility towards tazobactam-piperacillin, ertapenem and meropenem hence are potential antibiotics for empirical therapy. *Proteus* spp. was isolated mostly from urine, pus and tissue. Urinary tract infection was the type of infection that commonly associated with *Proteus* spp.

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REFERENCES

Bahashwan, S. A. and El Shafey, H. M. (2013). Antimicrobial resistance patterns of *Proteus* isolates from clinical specimens. *European Scientific Journal September* **9(27), 188-202.**

CDC. (2010). Laboratory Detection of Imipenem or Meropenem resistance in Gram-negative organisms. [Online] Available from: http://www.cdc.gov/hai/settings/lab/lab_imipenem.htmL (Accessed on 30 January 2017).

CLSI. (2015). Performance standards for antimicrobial susceptibility testing; approved standard; 25th informational supplement. CLSI document M100-S25. Clinical and Laboratory Standards Institute, Wayne, Philadelphia.

Donnenberg, M. S., Bennett, J. and Dolin, R. (2011). Mandell, Douglas and Bennetts's Principle & Practice of Infectious Diseases. 7th edn. Churchill Livingstone. Philadelphia. **pp. 2580.**

Feglo, P. K., Gbedema, S. Y., Quay, S. N. A., Adu-sarkodie, Y. and Opoku-Okrah, C. (2010). Occurrence, species distribution and antibiotic resistance of *Proteus* isolates: A case study at the Komfo Anokye Teaching Hospital (KATH) in Ghana. *International Journal of Pharmaceutical Sciences and Research (IJPSR)* **1(9), 347-352.**

Miriagou, V., Papagiannitsis, C. C., Tzelepi, E., Bou Casals, J., Legakis, N. J. and Tzouvelekis, L. S. (2010). Detecting VIM-1 production in *Proteus mirabilis* by an Imipenem-Dipicolinic Acid double disk synergy test. *Journal of Clinical Microbiology* **48 (2), 667-668.**

Mutnick, A. H., Turner, P. J. and Jones, R. N. (2002). Emerging antimicrobial resistances among *Proteus mirabilis* in Europe: Report from the MYSTIC program (1997-2001). *Journal of Chemotherapy* **14(3), 253-258.**

Neuwirth, C., Siébor, E., Duez, J. M., Péchinot, A. and Kazmierczak, A. (1995). Imipenem resistance in clinical isolates of *Proteus mirabilis* associated with alterations in penicillin-binding proteins. *Journal of Antimicrobial Chemotherapy* **36(2), 335-342.**

Newman, M. J., Frimpong, E., Donkor, E. S., Japheth, A., Opintan, J. A. and Asamoah-Adu, A. (2011). Resistance to antimicrobial drugs in Ghana. *Infection and Drug Resistance* **4(21), 215-220.**

O'Hara, C. M., Brenner, F. W. and Miller, J. M. (2000). Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. *Clinical Microbiology Reviews* **13(4), 534-546.**

Orrett, F. A. (1999). Prevalence of *Proteus* species in urinary tract infections in a regional hospital in Trinidad. *Zhonghua Yi Xue Za Zhi (Taipei)* **62, 438-442.**

Pal, N., Sharma, N., Sharma, R., Hooja, S. and Maheshwari, R. K. (2014). Prevalence of multidrug (MDR) and extensively drug resistant (XDR) *Proteus* species in a tertiary care hospital, India. *International Journal of Current Microbiology and Applied Sciences* **3(10), 243-225.**

Pavez, M., Vieira, C., de Araujo, M. R., Cerda, A., de Almeida L. M., Lincopan, N. and Mamizuka, E. M. (2016). Molecular mechanisms of membrane impermeability in clinical isolates of Enterobacteriaceae exposed to imipenem selective pressure. *International Journal of Antimicrobial Agents* **48(1), 78-85.**

Reslinski, A., Gospodarek, E. and Mikucha, A. (2005). Prevalence of multi-drug resistant *Proteus* species in clinical specimens and their susceptibility to antibiotics. *Medycyna Doswiadczalna I Mikrobiologia* **57(2), 175-184.**

Wang, J. T., Chen, P. C., Chang, S. C., Shiau, Y. R., Wang, H. Y., Lai, J. F., Huang, I. W., Tan, M. C. and Lauderdale, T. L. (2014). Antimicrobial susceptibilities of *Proteus mirabilis*: A longitudinal nationwide study from the Taiwan surveillance of antimicrobial resistance (TSAR) program. *BMC Infectious Diseases* **14, 486.**

Yah, S. C., Egbanfona, N. O., Oranusi S. and Abouo, A. M. (2001). Widespread plasmid resistance genes among *Proteus* species in diabetic wounds of patients in Ahmadu Bello University Teaching Hospital (ABUTH) Zaria. *African Journal of Biotechnology* **6(15), 1757-1762.**