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A MOLECULAR AND CLINICAL STUDY OF PROTEUS MIRABILIS ISOLATED FROM HUMAN

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Abstract:

To the family Enterobacteriaceae, Proteus mirabilis belongs as a Gram-negative bacterium. It causes a wide range of infections, including those of the genitourinary system, the respiratory system, the skin, and diabetic foot ulcers. Antibiotic-resistant bacteria are becoming more commonplace around the world. Proteus mirabilis has been found to be highly resistant to many antibiotics, which raises the risk of the development of multidrug resistance and treatment failure. In this investigation, Proteus mirabilis isolates were recognized using conventional biochemical techniques. The sensitivity test were used to determine antibiotic resistance while the PCR tests were performed with primers that were designed to bind to the ureR gene, which encodes the urease enzyme that is a virulence factor of P. mirabilis. All Proteus mirabilis isolates tested positive for the ureR gene, and the resulting amplicon length was 225 base pairs. We collected swabs from several body sites, including urine, wounds, burns, genitalia, ears, eyes, and sputum. The tetracycline showed 100% resistance. Toxicity to tetracycline, ampicillin, ampicillin-sulbactam, and sulfamethoxazole-trimethoprim was extremely high. The antibiotics cefepime, cefotaxime, ceftazidime, and cefoperazone were all shown to have intermediate resistance. The levels of resistance to piperacillin, amikacin, and aztreonam were rather low. 87.2% of the isolates showed MDR (multidrug resistance). The development of antibiotic-resistant microorganisms is directly attributable to the careless overuse of these drugs. Patients' access to antibiotics should be restricted by strict restrictions. Antibiotics can't be given out until susceptibility testing has been completed. Furthermore, our findings suggest that the ureR and based PCR method is a valid technique for characterizing Proteus mirabilis.

Keywords: Proteus mirabilis, molecular, human.

Introduction

Proteus is a member of the enterobacteriaceae genus (1). There are a total of eight species of Proteus, five of which have been given names and three of which have only been given their genomospecies (2). Gram-negative rod-shaped facultative anaerobic motile urease-splitting

bacterium (3, 4). Does not form capsules or spores. Urinary tract infections, burns, skin infections, eye infections, ear infections, nose infections, respiratory tract infections, and gastroenteritis are only some of the opportunistic nosocomial diseases that can be caused by these organisms if given the right conditions (4). Adhesion, swarming motility, urease, hemolysin, proteases, and lipopolysaccharide endotoxins are just some of the virulence components involved in pathogenesis that Proteus expresses. The synthesis of the enzyme urease is a hallmark of the genus Proteus and contributes significantly to its pathogenicity. Also, several different bacterial species, including Proteus species, have had gene clusters encoding this enzyme cloned. Three structural genes, ureA, ureB, and Urease C (ureC), as well as four auxiliary genes, ureD, ureE, ureF, and ureG, and a positive transcriptional activator, ureR, make up the urease gene cluster. Genes on the ure operon (ureA, ureB, ureC, ureD, ureE, ureF, ureG, and ureR) control urease enzyme synthesis. ureC has been identified as a key gene by previous research; this gene encodes the big subunit responsible for urease enzyme production in Proteus; because this subunit is largely conserved among all species, it is used as a diagnostic hallmark of this bacteria (8, 9). Urease structural gene transcription is urea inducible, with ureR serving as a regulator of urease activity to increase gene expression when urea is present (10).

As a serious global health problem, antibiotic resistance severely limits available treatment choices. Antibiotic resistance includes phenomena such as bacterial enzyme-mediated inactivation of antibiotics, reduced antibiotic uptake by bacteria, antibiotic efflux, and target-site mutation (11) are all ways that bacteria can develop resistance to antibiotics. In addition, bacteria can acquire multidrug resistance (MDR), which can render antibiotic treatment ineffective and contribute to the spread of persistent illnesses (12). PurposeWe hope to learn more about the antibiotic resistance profile of *P. mirabilis* by analyzing strains isolated from patients with urinary tract infections, diabetic foot ulcers, respiratory tract infections, burns, and wounds.

Materials and Methods

Patients from Al zahraa Hospital in Wasit province provided a total of 350 samples for bacterial isolation and identification. Distribution of acquired samples is displayed in (Table 1). Standard microbiological techniques were used for specimen treatment and isolation, and the isolated bacteria were identified via Gram staining, colony morphology, and biochemical testing. (13). Antibiotic resistance was determined using the Kirby-Bauer disc diffusion method (4). Using Muller- Hinton agar (Oxoid, Hamphsire, England) overnight cultures, bacterial suspensions were made. Approximately 1.5 x 108 CFU/mL (0.5 McFarland) was used to normalize the density of the suspensions. Using a sterile swab, the Suspensions were used to evenly inoculate the surface of a plate of Muller-Hinton agar. The plates were dried before the antibiotic discs were placed on them. After incubating the plates at 37 degrees Celsius for a full day, the widths of inhibition zones around the disks were recorded. UreR gene was used to conduct a molecular analysis on all P. mirabilis isolates. All of the bacterial isolates were processed in accordance with the manufacturer's instructions for the DNA extraction kit (GeNet Bio, Korea).

Measurements of DNA concentration and purity were made using a Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) set to absorbance (260/280 nm) in accordance (14) The lyophilized primers were supplied by GENEXIZ (South Korea). Using forward primer sequences (5'-GGTGAGATTTGTATTAATGG-'3) and reverse primer sequences (5'ATAATCTGGAAGATGACGAG-'3), a PCR was performed (15) to identify P. mirabilis based on the ureR gene, yielding a 225 bp result. Electrophoresis of PCR results in a 1.2% agarose gel stained with a non-toxic dye (Biolabs-USA) was used to determine their quality. We employed a UV Trans-illuminator (San Gabriel, USA) to check out the DNA bands.

Result

The results were interpreted according to Clinical Laboratory Standards Institute guidelines.

Table 1: Source and frequency of *P. mirabilis* isolates

Source	**No. of samples**	**No. (%) of *P.mirabilis* isolates**
Urine samples	115	18 (15.6%)
Surgical wound swabs	110	9 (8%)
Diabetic foot swabs	50	7 (14%)
Endotracheal aspirate samples	50	6 (12%)
Burn swabs	25	3 (12 %)
Total	350	43 (12.2 %)

Table 2 displays the antibiotic resistance profile, which revealed a wide range of resistance levels. Tetracycline induced a 100% level of resistance. The ampicillin and ampicillin-sulbactam combinations showed high resistance (85.1% each), as did the sulfonamides trimethoprim and chloramphenicol (78.8% and 72.2%, respectively). Among the antibiotics tested, intermediate resistance was found for 53.2% of cefepime, 51.1% of cefotaxime, 44.7% of ceftazidime, 42.6% of cefoperazone, 42.6% of gentamicin, 38.3% of ciprofloxacin, and 31.9% of levofloxacin. Piperacillin and amikacin both had 25% resistance, whereas aztreonam had 14.9%, imipenem had 8.5%, and meropenem had 6.4%. Table 3 displays the frequency of *Proteus mirabilis* MDR isolates. Overall, 87.2% of the tested isolates were MDR.

Table 2: The susceptibility of *Proteus mirabilis* isolates to several antibiotic disks is analyzed here.

Antibiotic disks No of resistant isolates (%)	**No of resistant isolates (%)**
Ampicillin	40 (85.1)
Piperacillin	12 (25.5)
Ampicillin-sulbactam	40 (85.1)
Tetracycline	43 (100)
Cefotaxime	24 (51.1)
Cefoperazone	20 (42.6)
Ceftazidime	21 (44.7)
Cefepime	25 (53.2)
Aztreonam	7 (14.9)
Amikacin	12 (25.5)
Sulphamethoxazole-trimethoprim	37 (78.8)

All *P. mirabilis* isolates tested positive for the presence of the ureR gene at 225bp, as shown in Figure (1), with the exception of isolates 16, 31, 35, and 44, which resulted in ambiguous bands on agarose. Figure 4 displays the findings of a second round of gel electrophoresis performed on these isolates. Positive PCR results for the ureR gene (225 bp) were seen on gel electrophoresis in 100% of *P. mirabilis* isolates (figure 1).

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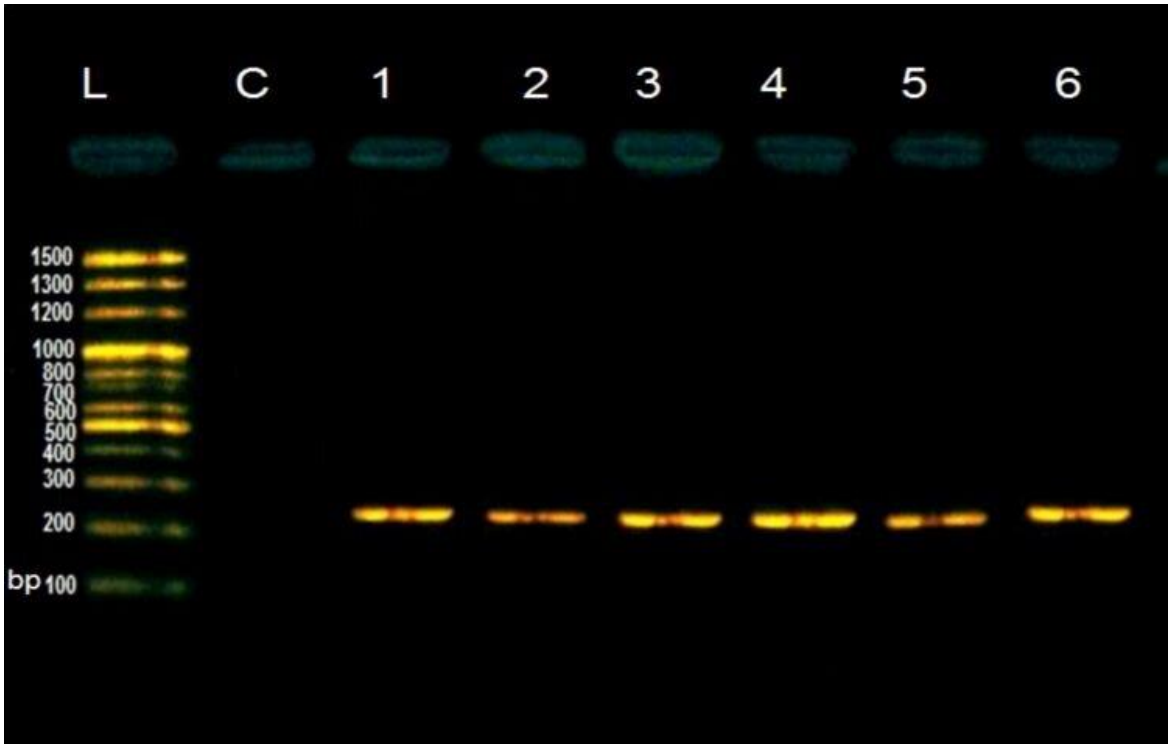

Figure 1: PCR product of amplified ureR gene of *P. mirabilis* isolates (amplicon with 225bp) electrophoresed on Agarose (1.2%) gel. DNA marker (100 bp) in lane M; *P. mirabilis* amplicon sequences in lanes 1–7.

Discussion

Among the many illnesses that the opportunistic bacteria *Proteus mirabilis* can cause, urinary tract infections are by far the most common (5). In addition, *Proteus mirabilis* is a prevalent cause of diabetic foot ulcers (15). When it penetrates a cut or the respiratory system, it can cause disease (13). The goal of this study was to examine whether or not the *P. mirabilis* strain used was resistant to antibiotics. This study found a prevalence of 12.2% for *Proteus miabilis* after isolating 43 individuals. Prevalence rates of 12.4% and 13.2% were observed, which are similar with what was indicated (16, 17). Studies have indicated prevalence rates as low as 2% and as high as 5.2% (19, 4). One hundred percent of the recovered isolates exhibited high levels of resistance to tetracycline, which is in line with reports of 100% resistance from (17), but lower than the 85% and 82% rates described elsewhere (4, 19). Between 72% and 85% of the bacteria we tested were resistant to the antibiotics ampicillin, sulfamethoxazole, and chloramphenicol. Resistance to ampicillin, sulbactam, and trimethoprim ranged between 78% and 82%, which is in line with the findings of another investigation (4). Ciprofloxacin resistance was found to be substantially higher (51%). It is utilized with just a 26.66% chance of success. Our results suggest that some medications are not appropriate for use against P.mirabilis infections, and we discuss why. The resistance rates seen for ceftazidime (40%) and ceftotaxime (30%) were both higher than those observed in (22). Additional resistance to

cefepime was found in a higher number of isolates (17) than had been previously reported. On the other side, it was found that resistance was lower than normal (82.4%). There was a moderate degree of resistance (> 30%) among *Proteus mirabilis* isolates to piperacillin, amikacin, and aztreonam. Imidacloprid and ertapenem had the lowest rates of resistance (8.5% and 6.4%), as determined by Adamus-Bialek's (23), testing of all antibiotics. The urease gene, (ureR), which is responsible for the production of urease enzyme and is regarded a diagnostic characteristic of these species, was amplified from *P. mirabilis* isolates using PCR with species-specific primers in this study. *Proteus mirabilis* infections are characterized by the presence of urease, one of the most critical virulence agents of *P. mirabilis*. Scientific studies have repeatedly demonstrated the high urease production capacity of *P. mirabilis* species (24). Urea is created in the human body after the breakdown of amino acids and is found everywhere. Urease is an enzyme that helps Proteus spp. use urea as a nitrogen source by converting it to ammonia and carbon dioxide. Disruption of host function and direct tissue damage at infection sites are known outcomes of this process, which leads to an elevated local pH (25). Several publications (15) have examined the use of a ureR-based molecular approach for identifying *P. mirabilis*, and they all agree that using a species-specific primer based on the conserved ureR sequence of *P. mirabilis* results in an amplified DNA product of 225 bp that can be spotted on an agarose gel. *P. mirabilis* can be identified with PCR because to ureR's high discriminatory power (26). Using 1% agarose gel electrophoresis, we found that all *P. mirabilis* isolates from UTI patients carried the ureR gene that encodes for urease, as indicated by the presence of an amplicon with a molecular weight of 359 bp (27). Each *P. mirabilis* isolate tested positive for ureR at a size of 225bp when amplified using PCR with ureR-specific primers (28). In accordance with the results, ureR amplicon products of 225 bp were obtained from all of the *P. mirabilis* isolates studied.

Conclusion

This research demonstrates that a 250bp PCR approach based on ureR can be employed for the targeted detection of *P. mirabilis*. Proteus bacteria may develop resistance to antibiotics for a number of reasons. Education on antibiotic usage and misuse, stringent prescription limits, and susceptibility testing prior to antibiotic administration are all necessary to combat the rise of antibiotic-resistant microorganisms.

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