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Research Article

INCIDENCE OF AMINOGLYCOSIDES-RESISTANT *ROTHIA MUCILAGENOSA* CAUSING RESPIRATORY INFECTIONS IN WORKERS OF AL-BAIJI OIL REFINERY, IRAQ

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ABSTRACT
Rothia mucilaginosa was isolated from 7 patients working in Al-Baiji oil refinery suffering from respiratory tract infections during 2008to 2009. The infections included five cases of tonsiilitis and bronchitis and pneumonia once each. The various strains were cultured from specimens obtained by swabs and sputum specimens. They were identified largely depending upon the determination of the Mol% of G+C content of DNA which was high as 57%, 58.8% and 60.0% (average 58.8%). All strains were fully susceptible to vancomycin and cefotaxime. They showed variable susceptibility to tetracycline, ciprofloxacin, ceftriaxone amd amoxicillin/clavulanic acid. A high resistance to tobramycin, amikacin and gentamicin was seen with MICs ranged as 8, 0.125-16 and 0.5-16 µg/ml respectively.
Key words: *Rothia mucilaginosa*, aminoglycosides resistance, Al-Baiji oil refinery, Iraq.

INTRODUCTION

Most of the surfaces of the upper respiratory tract are colonized by normal flora. Some of these organisms are opportunistic causing infection among immune deficient individuals. Bacterial infections of respiratory system might be increased as a result of health conditions such as allergies, viral infections, smoking and airborne environmental pollutants[2]. Almost all refineries cause unacceptable environmental chemical pollution[2]. Oil refineries are the largest sources of air pollution with large amounts of emissions include heavy metals, dust particles, gases like sulfur dioxides (SO_2), nitrogen oxide (NO_2), carbon dioxide (CO_2), carbon monoxide, methane, dioxins, hydrogen fluoride, chlorine, benzene and others[3]. Throughout the refining process. Workers can be potentially exposed to various health hazards related to these pollutants[3]. Air pollution principally affects the respiratory, circulatory and olfactory systems. The respiratory system is being the main route of enty for air pollutants. Pollutants inhaled in the workplace can lead to all the major chronic lung diseases except those due to vascular illnesses[2]. Exposure to environmental pollutants may lead to selective pressures favoring certain organisms or genotypes. Recent studies demonstrated high correlations between industrial pollution and the spatial distribution of antibiotic resistance[4]. Antibiotic resistance in medicine generates significant health and economic impacts [3]. Heavy metals such as lead, mercury, cadmium and nickel are emitted from oil refinery systems are metabolically poisonous at low concentrations. Bacterial resistance to toxic heavy metals is a widespread phenomenon. The mechanisms of heavy metal resistance have been reported to enhance the antibiotic resistance ability of microorganisms [5]..*Rothia mucilaginosa*,formerly classified as *Stomatococcus mucilaginosus* [6] is a gram positive, coagulase-negative, non-spore forming cocci considered as a part of the commensal flora of the oral cavity and the upper respiratory tract of man [6]. There is an increasing number of infections reported for *R. mucilaginosa* especially among immunocompromised patients[7,8]. This organism has been received an attention recently as a potential emerging opportunistic pathogen. The virulence factors made this organism as an opportunistic pathogen or as a member of the health hazard oral microbiota are mostly still obscure. The present study was an attempt for identification of aminoglycosides-resistant R. mucilaginosa which is locally unknown as a respiratory pathogen associated with oil refinery pollution.

MATERIALS AND METHODS

Patients

Patients samples were taken from upper and lower respiratory tract of patients with respiratory infections during 2008 to 2009. 200 samples were collected from workers of the Northern Refineries Company, the largest establishment in the country, which is located in the North of Al-Baiji town, Salahldeen Provence. This factory is divided into several major sub-sections, and the section of production is one of those where the workers exposed to the direct and indirect risks of pollution. Equal number of samples was taken from patients attended Tikrit teaching hospital as control.

Identification Methods

Bacterial isolates were identified using conventional methods which confirmed by the Mol% of G+C content of DNA.

Determination of G+C %

Extraction of genomic DNA: Genomic DNA was extracted from unknown bacteria. A standard strain of *Escherichia coli* mj83 (the mol% G + C of the DNA is: 50.9) was used as control by the salting-out method of Pospiech and Neumann [8] modified by Kieser[2].

Estimation of DNA concentration

The concentration was calculated from the optical density at 260 nm by utilizing UV-Visible Spectrophotometer (Aquarius, Germany) and distilled water as a control solution according to the following equation:
Unknown µg/ml = 50 µg/ml x Measured A260 nm x Dilution factor [9].

Determination of DNA purity

Purity of isolated DNA was measured by dividing the absorbance value of diluted sample at 260 nm on the absorbance value at 280 nm [10].

Determination of Tm

DNA was carefully dispersed in distilled water and the thermal denaturation curve was determined stepwise by using an adjustable water bath. The temperature of the DNA solution was measured by using a thermometer placed in the liquid. A few temperature measurements were made to reach the melting temperature (Tm) [11]. The %G+C was calculated from the equation:

%G+C = 2.44 (Tm- 69.4) [12].

Antibiotic Susceptibility Testing

The disc diffusion method was used to determine antibiotic sensitivity of the isolates[5]. Overnight broth cultures were spread on Mueller Hinton agar plates. As a fastidious microorganisms, 5 % defibrinated human blood was added to the medium The plates were dried at room temperature for 2 h. Antibiotic discs were placed at equi-distances and incubated for 24 h at 37"C .The organisms were classified as sensitive, intermediate or resistant, based on the NCCLS standards. A total of 3 aminoglycosides were used.

Determination of MIC of Antibiotics

The agar dilution susceptibility-testing method was utilized for the determination of the minimal inhibitory concentration (MIC) of aminoglycosides tested[13].

RESULTS

Strains characteristics

Seven cases (3.5%) only of respiratory infections were noticed among the refinery patients samples revealed *Rothia mucilaginosa* that microscopically appeared as pairs and tetrads which were encapsulated when stained with cengo red. They were facultative anaerobic. Colonies on blood agar were almost similar to the colonies of *Staphylococcus spp.* But they were catalase negative and did not grow on mannitol salt agar(Table1). The mol% of G+C values was high as 57.6%, 58.8% and 60.0% (average, 58.8%) which showed that these strains belonged to the genus *Rothia* , species *Rothia mucilagenosa*, formerly *Stomstococcus mucilagenosus.* This pathogen caused tonsillitis in five cases and the other two strains caused bronchitis and pneumonia.

Antibiotic resistance

Table 2 shows that all strains of *R. mucilagenosa* were sensitive to vancomycin and cefotaxime, and highly sensitive to ceftriaxone, amoxicillin/clavulanic, ciprofloxacin and tetracycline. While high resistance to tobramycin, amikacin and gentamicin was seen with MICs ranged as 8µg/ml, 0.125-16 µglml and 0.5-16µg/ml respectively.

Table 1: Characteristics OF *Rothia mucilaginosa* strains

Characteristic	Result
Cell arrangement	Cluster ,diplococci
Capsule	+
Catalase	+
Growth on NA agar with 5% NaCl	-
VP test	+
Anaerobic growth	+
Glucose fermentation	+
Fructose fermentation	+
Trehalose fermentation	+
Maltose fermentation	+
Mannitol fermentation	_
Rhafnose fermentation	_
G+C content (mol%)	57.6,58.8,60.0

Symbols:+,positive reaction;-,negative reaction; NA, nutrient agar.

Table 2: Antibiogram of *R. mucilaginosa* Amikacin, (AK30); Gentamicin, (CN10); Tobramycin, (TOB10)

Antibiotic	No.(%) resistant strains by disc diffusion	MIC of total strains(µg/ml)	MIC of resistant strains(µg/ml)
Amikacin(AK)	4(57.1%)	0.125-16	8-16
Gentamicin(CN)	4(57.1%)	0.5-16	16
Tobramycin(TOB)	7(100%)	8	8

DISCUSSION

R. mucilaginosa is considered as a part of the commensal flora of the oral cavity and the upper respiratory tract in humans[7]. It was reported an increasing number of infections due to *R. mucilaginosa*, especially among immunocompromised patients[7]. It was isolated elsewhere from 8 patients suffering from lower respiratory tract infections over a 4-y period (1999-2003) and the infections ranged from mild cases of pneumonia to a life-threatening case of recurrent lung abscesses[14]. Apart from *R mucilaginosa* no other pathogens were found to account for such infection[7]. Moreover, A study was carried out by Klein-Petal, and his co-workers[15] proved that there was an association between poor ambient air quality and increased morbidity and mortality, including respiratory infections. They showed the effect of several air pollutants, such as particulate matters on antimicrobial mechanism of host defense in the airway. However, it has been known that low doses of residual oil fly ash like vanadium oxides and vanadium sulfates inhibit the ability of airway epithelial cells to respond to inflammatory stimuli and suggested that exposure to these agents could result in an impairment of defense against pathogens. The impact of several pollutant gases emitted in high and moderate constant levels on the immune system, especially on the respiratory immune response of oil refinery workers that intentionally exposed might be enhanced the opportunistic normally found bacteria to invade and cause infection [15]. On the other hand, resistance genes can be taken up by indigenous bacteria and spread by mechanisms of genetic transfer. Examples of resistance genes originating in commensal or environmental bacteria and transferring to pathogens have previously been described [16]. This result from the inherent genetic fluidity of bacteria. In refinery environment, many heavy metals may be emitted as oil byproducts and many of these still in the air as Particulate matter (PM) emissions. According to Huffinan, et al[17], these particulate matters largely composed of Ni, V, Fe, Cu, Zn, and Pb sulfates. Particulate matters easily penetrate into respiratory airways. This makes bacteria with direct exposure to heavy metal and may develop a pattern of heavy metal resistance as a response to selective pressure. However, It is well known that there is a correlation between the resistance to some heavy metals and the resistance to antibiotics, because genes for antibiotic and heavy metal resistance have been located both on plasmids, tansposons and on the chromosome. Furthermore, aminoglycosides are locally highly and sometimes blindly used to overcome the resistant bacteria. Abuse antibiotics with refinery chemical pollution might cause an emergence of highly aminoglycosides-resistant bacteria that can be locally isolated from man. The present are in support of earlier suggestions that combined expressions of antibiotic resistance and metal tolerance may

not be a chance phenomenon but rather the results of selection by metals present in an environment. Thus, the intensive pollution in this refinery might also contribute to the selection of antibiotic resistance genes in *R. mucil aginosa that* colonizes humans.

CONCLUSION

The impact of several pollutants in high and moderate constant levels on the immune system, especially on the respiratory immune response of oil refinery workers that intentionally exposed, might be enhanced normally found *R. mucilaginosa* to invade and cause infection. Exposure to environmental pollutants may lead to selective pressures favoring certain organisms or genotypes. Multi-resistance patterns to aminoglycosides were cornnon among the refinery isolates of *R. mucilaginosa* and the results indicate that this phenomenon might be attributed to the pollutants emitted during oil refining processes and misuse of these antibiotics.

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