Bacterial Contamination of Indoor Air in Schools of Riyadh, Saudi Arabia

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Abstract

**Background:** The presence of bacteria in the indoor air poses a serious problem from the point of view of health protection and environmental engineering. Precise determination of various groups of bacteria indoors is necessary for both to estimate the health hazard and to create standards for indoor air quality control. This is especially important in such densely populated facilities like educational institutions.

**Results:** In the present study, 252 samples of indoor air and air conditioners of 25 schools (Middle Schools) Public and Special, those were collected from east and centre of Riyadh in, Saudi Arabia, between October and November 2015. The most common bacterial isolates were, Gram negative bacteria (Pseudomonas stutzeri, Francisella tularensis and Ralstonia mannitolilytica ) of the total airborne bacteria. Whereas, the prevailing Gram positive bacteria were the Staphylococcus aureus, followed by Kocuria kristinae. Also, Pseudomonas stutzeri was isolated from the air conditioners of special schools. Results revealed that the highest level of bacterial contamination was detected in the class rooms during lessons.

**Conclusions:** High concentrations of bacteria was observed in the public schools (where overcrowded classrooms). Results obtained in this study showed that the bacterial species varied in their susceptibility to all the antimicrobials used. Majority of them showed resistance to one or more of antimicrobials compounds. It was observed that Ciprofloxacin was the most effective antibiotic to all bacterial species used during this study.

**Keywords:** Bacterial; Contamination; Air; School; Antibiotic susceptibility

**Introduction**

Millions of children and adults across the nation spend their days in school buildings, and they need safe, healthy environments to thrive, learn, and succeed [1]. Indoor air quality has become an important public health concern as most people spend more than 90% of their time in indoors like houses, offices and schools [2]. Air in the indoor environment can be polluted by a number of pollutants among which airborne microorganism (bacteria and fungi) are one of the most important. It has been estimated that one-third of indoor air quality (IAQ) complaints may be due to microbial contamination [3] and exposure to these may cause allergies, respiratory and immunotoxic diseases [4]. Several investigators have reported these contaminants in different indoor environments such as schools and hospitals [5-8]. Schools are public places inhabited by thousands of students every day and tend to have high levels of activity that typically result in higher levels of airborne fungi and bacteria [6]. Biological contamination of indoor air is mostly caused by bacteria, moulds and yeasts. They can be dangerous as pathogenic living cells but they can also secrete some substances harmful for health [9]. These are different kinds of toxic metabolism products, for example mycotoxins [10-12]. The amount of the microbial content of indoor air of school is an important parameter because it has a direct impact on the mental health [13], physical development and performance of the students. Epidemiological studies show that high concentration of microorganisms in the air can be allergenic; however, sometimes even very low concentrations of some particular microorganisms can cause serious diseases [14]. Childhood asthma is one of the commonest chronic respiratory disorders in Asian countries [15-18] also noted that allergic disease (nasal allergy, asthma, and other allergies) is the number one chronic childhood illness. The conditioning systems are to provide occupants with a more comfortable environment. Nevertheless, such artificial environments may be favorable to fungi, bacteria, protozoans and mites growth, which may bring health risks to users, either by hypersensitivity or infections [19-22]. According to the American Society of Heating, Refrigerating and Air Conditioning Engineers (ASHRAE, 2000), the health effects caused by microorganisms that are in indoor environments with air conditioning systems can be infective or immunological. Diriba L et al. [23] revealed that Lack of cleaning and checking out of the heating, ventilation and air conditioning systems (HVAC) may allow microbial growth, which causes rhinitis, bronchitis, pharyngitis, pneumonia, conjunctivitis and keratitis in the users. Several studies have occurred on antibiotic susceptibility of indoor airborne bacteria isolated from selected places have done by [24,25] and others.

The objective of the present study is qualitative determination of airborne and air conditioner bacterial contamination at school classrooms of different indoor schools at Riyadh and to study the effect of several antibiotics on the cultures of isolated bacterial species.

**Materials and Methods**

Samples Collection: 252 samples of 25 schools (Middle Schools)* Public and Special, that were collected from east and centre of Riyadh in, Saudi Arabia between October and November 2015 (Table 1). Collecting of the samples was conducted in the autumn during this season, the percentage of moisture environmental content was relatively between 21% and 36% during October and November and the temperature degree was relatively between (41°C- 15.4°C). The...
Microbial identification

Objective: The VITEK 2 is an automated microbial identification system that provides highly accurate and reproducible results (VITEK 2) (Figure 1).

Reagent cards: The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalization, enzyme hydrolysis, and growth in the presence of inhibitory substances. An optically clear film present on both sides of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vessel that prevents contact with the organism-substrate admixtures. Each card has a pre-inserted transfer tube used for inoculation. Cards have bar codes that contain information on product type, lot number, expiration date, and a unique identifier that can be linked to the sample either before or after loading the card onto the system. Figure 2 shows the GN card (VITEK 2).

There are currently four reagent cards available for the identification of different organism classes as follows:

1. GN - Gram-negative fermenting and non-fermenting bacilli
2. GP - Gram-positive cocci and non-spore-forming bacilli
3. YST - Yeasts and yeast-like organisms
4. BCL - Gram-positive spore-forming bacilli

Culture requirements: The on-line product information contains a culture requirements table that lists parameters for appropriate culture and inoculum preparation. These parameters include acceptable culture media, culture age, incubation conditions, and inoculum turbidity (VITEK 2).

Suspension preparation: A sterile swab or applicator stick is used to transfer a sufficient number of colonies of a pure culture and to suspend the microorganism in 3.0 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12 × 75 mm clear plastic (polystyrene) test tube. The turbidity is adjusted accordingly (Table 1) and measured using a turbidity meter called the DensiChek™ (VITEK 2) (Table 1a).

Inoculation: Identification cards are inoculated with microorganism suspensions using an integrated vacuum apparatus. A test tube containing the microorganism suspension is placed into a special rack (cassette) and the identification card is placed in the neighbouring slot. The vacuum is applied and air is re-introduced into the station, the organism suspension is forced through the transfer tube into micro-channels that fill all the test wells (VITEK 2).

Incubation: The carousel incubator can accommodate up to 30 or up to 60 cards. All card types are incubated on-line at 35.5 ± 1.0°C. Each card is removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next read time. Data are collected at 15-minute intervals during the entire incubation period (VITEK 2).

Using the Biomérieux Vitek™ 2 system automated antimicrobial susceptibility testing systems (Figures 3 and 4)

Optical system: A transmittance optical system allows interpretation of test reactions using different wavelengths in the visible spectrum. During incubation, each test reaction is read every

### Table 1: Kind of schools and numbers of bacterial isolated samples.

| Kind of school | Number of isolates |
|---------------|--------------------|
| BA            | 22                 |
| NA            | 2                  |
| BA and NA     | 22                 |

### Table 1a: Suspension turbidities used for card inoculation.

| Product | McFarland Turbidity Range |
|---------|--------------------------|
| GN      | 0.50-0.63                |
| GP      | 0.50-0.63                |
| YST     | 1.80-2.20                |
| BCL     | 1.80-2.20                |
Figure 2: VITEK 2 GN colorimetric identification card.

Figure 3: VITEK 2 compact cassette loaded with 10 cards and suspension tubes and bar code scanner for data entry.

Figure 4: VITEK 2 cassette loaded with cards and suspension tubes being loaded into the automatic transport system.

15 minutes to measure either turbidity or colored products of substrate metabolism. In addition, a special algorithm is used to eliminate false readings due to small bubbles that may be present.

Antimicrobial screen: We have used a range of ESBL, Ampicillin, Ampicillin/Subbactam, Piperacillin, Cefazolin, Cefoxitin Cefazidime, Ceftriaxone, Ceftepim, Ertapenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Levofoxacin, Nitrofurantoin, Trimethoprin/Sulfamethoxazole, Cefoxitin, Benzylpenicillin, +Amoxicillin, +Ampicillin, +Ampicillin/Clavulanic acid, Piperacillin/Tazobactam, Oxacillin, +Cefaclor, +Cefradine, +Cefuroxime, +Cefdinir, +Cefixime, +Cefotaximicillin, +Cefaclor, +Cefradine, +Cefuroxime, +Cefdinir, +Cefixime, +Cefotaxime, +Ceftazidime, +Ciprofloxacin, Moxifloxacin, InducibleClindamycin Resistance, +Clarithromycin, Erythromycin, Clindamycin, Linezolid, Teicoplanin, Vancomycin, +Doxycycline, Tetracycline, Tigecycline, Fosfornycin, Nitrofurantoin, Fusidic Acid, Mupirocin, Rifampicin, +Trimethoprin, Trimethoprim/Sulfamethoxazole, Augmentin, Cefotaxime 3rd, Ciprofloxacin, Doxycycline, Amoxicillin, Cefuroxime, Norfloxacin, Trimethoprim-Sulfame, Cefixime 3rd, Azithromycin, Clindamycin.

Special Middle schools have Three grades each grade have Two classes.

Results and Discussion

From the present study 252 samples of indoor air and air conditioners of 25 schools (Middle Schools) Public and Special, that was collected from east and centre of Riyadh in, Saudi Arabia between October and November 2015 (Table 1). Collecting of the samples were conducted in the autumn during this season, the mean humidity value was relatively (21% and 36%) in October and November respectively, with the decrease in the environmental temperature, due to the use of air conditioners. The minimum temperature degrees were (20.9°C - 15.4°C) and the maximum were (28.1°C - 21.4°C). Bouillard L et al. [27] found that the humidity recorded in the autopsy room was significantly low in the summer (because of air conditioner). The mean humidity value of the room at the time of the autopsy was 56%. It was determined that humidity had no effect on the amount of bacteria and fungi grown by two methods during each sampling season (p>0.05). The amount of the microbial content of indoor air of school is an important parameter because it has a direct impact on the mental health, physical development and performance of the students. Numerous studies have shown that occurrence of bacteria and moulds in the air of school poses a great risk to children [14,17,18,28,29]. In the present study the most common bacterial isolates were, Gram negative bacteria (Pseudomonas stutzeri, Francisella tularensis and Ralstonia mannitolyltica ) of the total airborne bacteria. Whereas, the prevailing Gram positive bacteria were Staphylococcus aureus, followed by Kocuria kristinae (Table 3). Pastuszka JS et al. [30] found that some bacteria, such as coagulase-negative Staphylococcus, Corynebacterium and Bacillus, were predominant (42.7%, 20.4% and 6.9% of the total, respectively) in the indoor air of primary schools in Edirne City, Turkey. While [25] observed that the bacterial profile of indoor air sample showed that S. aureus was the most frequently isolated species among potential pathogenic bacteria identified in both SW and OR units. But, in Chalachew G et al. [24] stated that Staphylococcus is the most frequently occurring bacteria in clinic/hospital rooms 44 followed by Micrococcus, which together contributed 58-78% of total bacteria concentration [31]. However, Bouillard L et al. [27] point out that the amount of normal bacterial flora, particularly such as CNS, diphteroid bacillus and Micrococcus spp., were increased with the number of staff present in the room. Microbiological indoor air quality of a school is an important factor for children's health, as school serve a daily environment for them. Microbial concentration of indoor air of the school is affected by many factors, including human activity, the age of school building, ventilation conditions, outdoor air, season (primarily temperature and humidity), etc. [13]. Strong relationship between occupant density, human activity and microorganisms concentration in
| Teacher | Student | Age | Neighborhood | Acute disease | Chronic disease | Students Numbers | Marital state | Children numbers |
|---------|---------|-----|--------------|---------------|-----------------|-----------------|--------------|-----------------|
| Public1 | √       | Between 11 – 16 | - AL-Fayha - AL-Saadah - AL-Jazeerah | - Sore throat - Stomach Ache - Cold - Fever - Tonsillitis - Flu - Cough - Headache - Dizziness | - Asthma - Eczema - Anemia - Urinary tract infection - Rheumatoid - chest allergy | Between 40 – 53 | - | - |
| Public2 | √       | Between 12 – 15 | - AL-Rawabi - AL-Salam - AL-Rayan - AL-Fayha - AL-Manar | - Cough - Flu - Cold - Headache - Tonsillitis - Eye allergy - Cough - Fever - Stomach Ache | - Asthma - Anemia - Diabetes | Between 30 – 45 | - | - |
| Special 1 | √      | Between 11 – 15 | - AL-Jazeerah - AL-Fayha - AL-Rawdah - AL-Salam - AL-Manar | - Cold - Sore throat - Flu - Fever - Cough | - Bronchitis - chest allergy - Heart disease - Diabetes - Asthma - Sinusitis | Between 16 – 22 | - | - |
| Special 2 | √      | Between 12 – 17 | - AL-Rawabi - AL-Rabwah - AL-Jazeerah - AL-Manar - AL-Salam - AL-Naseem | - Stomach Ache - Cold | - Diabetes - Eczema - Asthma - Anemia - Bronchitis - Diabetes - Blood Pressure - Palpitation | Between 13 – 20 | - | - |
| Special 3 | √       | Between 12 – 16 | - AL-Jazeerah - AL-Rawdah - AL-Manar - AL-Rawabi - AL-Rabwah | - Cold - Cough - Sore throat - Fever - Tonsillitis - Dizziness | - Asthma - Eczema - Anemia | Between 17 – 24 | - | - |
| Special 3 | -       | Between 12 – 16 | - AL-Jazeerah - AL-Rawdah - AL-Manar - AL-Rawabi - AL-Rabwah | - Cold - Cough - Sore throat - Fever - Tonsillitis - Dizziness | - Asthma - Eczema - Anemia | Between 17 – 24 | - | - |

Table 2: Most common disease in schools between teachers and students.
Table 3: Bacterial species isolated from the indoor air and air conditioners of five schools.

| No | Bacterial isolates         | Kind of school                      | Total No. of Samples |
|----|----------------------------|-------------------------------------|----------------------|
| 1  | Pseudomonas stutzeri       | Special (air)                       | 31                   |
| 2  | Pseudomonas stutzeri       | Special and public (air-conditioners) | 49                   |
| 3  | Kocuriakristinae           | Public (air)                        | 48                   |
| 4  | Francisella tularensis     | Public (air)                        | 52                   |
| 5  | Ratstonia mannitoltyca     | Special (air)                       | 26                   |
| 6  | Staphylococcus aureus      | Special (air) and public (air)      | 62                   |

| Bacterial species | Antimicrobial agents |
|-------------------|----------------------|
|                 | R. mannitolyltica    | F. tularensis | K. kistinae | S. aureus | P. stutzeri |
|                  | S/R* MIC            | S/R* MIC     | S/R* MIC   | S/R* MIC | S/R* MIC |
| R. mannitolyltica|                      |              |            |          |          |
| S                | 8 Ampicillin        |              |            |          |          |
| R                | 4 Ampicillin/Sulbactam |            |            |          |          |
| S                | =4< Piperacillin    |              |            |          |          |
| R                | =64< Cefazolin      |              |            |          |          |
| S                | =64< Cefoxitin      |              |            |          |          |
| S                | 2 Ceftriazone       |              |            |          |          |
| S                | =1< Ceftpim         |              |            |          |          |
| S                | 1 Meropenem         |              |            |          |          |
| S                | =2< Amikacin        |              |            |          |          |
| S                | =0.5<                |              |            |          |          |
| S                | =1< Gentamicin      |              |            |          |          |
| S                | =1< Tobramycin      |              |            |          |          |
| R                | =0.25< Ciprofloxacin |            |            |          |          |
| R                | 256 Nitrofurantoin  |              |            |          |          |
| S                | 40 Trimethoprin/Sulfamethoxazole |  |
| S                | + Cefoxin Screen    |              |            |          |          |
| R                | =0.5> Benzylipenicillin |            |            |          |          |
| R                | + Amoxicillin       |              |            |          |          |
| R                | + Ampicillin        |              |            |          |          |
| S                | + Ampicillin/Clavulanic Acid |  |
| S                | Piperacillin/Tazobactam |            |            |          |          |
| S                | =0.25< Oxacllin     |              |            |          |          |
| S                | + Cefaclor          |              |            |          |          |
| S                | + Cefradine         |              |            |          |          |
| S                | + Cefuroxime        |              |            |          |          |
| S                | + Cefdinir          |              |            |          |          |
| R                | + Cefixime          |              |            |          |          |
| S                | + Cefotaxime        |              |            |          |          |
| R                | + Ceftazidime       |              |            |          |          |
| S                | + Ciprofloxacin     |              |            |          |          |
| S                | =0.25< Moxifloxacin |              |            |          |          |
| -                | NEG Inducible Clindamycin Resistance |  |
| S                | + Clarithromycin    |              |            |          |          |
| S                | =0.25< Erythromycin |              |            |          |          |
| S                | =0.25< Clindamycin  |              |            |          |          |
| S                | 1 Linezolid         |              |            |          |          |
| S                | =0.5< Teicoplanin   |              |            |          |          |
| S                | =0.5< Vancomycin    |              |            |          |          |
| S                | + Doxycycline       |              |            |          |          |
| S                | =1< Tetracycline    |              |            |          |          |
| S                | =0.12< Tigecycline  |              |            |          |          |
| S                | =8< Fosfomycin      |              |            |          |          |
the indoor air was reported elsewhere [32-34]. Among the five schools that were included during this study, it was found that the highest bacterial concentration in heavily populated workplaces. Also, the highest level of bacterial contamination was detected in the class rooms during lessons. Relatively high concentrations of bacteria was observed in the public schools (where overcrowded classrooms). This study is in agreement with the work of, Li WM et al. [34] who suggests that the highest bacterial concentration in heavily populated workplaces. While Fleische RM et al. [32] states that in schools the highest level of bacterial contamination was detected in the corridor and in rooms. During lessons and after lessons the number of microorganisms was much lower. But, Carvalho CL et al. [35] evaluating many shopping centres in Hong Kong found different bacteria levels on the weekend when the number of people inside the building increased. However, Strzyakowska-Sekulska et al. [9] reported in their investigation just after the short period of high microorganism's concentration in the air of investigated rooms a gradual decrease of microbial contamination. The bacterial species isolated during this investigation from the indoor air of the special schools were Pseudomonas stutzeri, Raclistonia mannitolitica and Staphylococcus aureus, also Pseudomonas stutzeri was isolated from the air conditioners (Table 3). But, Kocuria kristinae and Francisella tularensis were isolated only from the indoor air of public schools. In the special schools the level of bacterial contamination was lower than public schools. Among the bacterial pathogens present in the tested classrooms there were Francisella tularensis, the causative agent of tularemia [36]. Strzyakowska-Sekulska et al. [9] Considered the analysis of bacterial flora composition in investigated University rooms revealed dominating contributions of bacteria from the following genera: Micrococcus spp, Bacillus spp, Staphylococcus spp. (e.g. Staphylococcus aureus), Sarcina spp, and Serratia spp. Also some gram negative bacteria belonging to Escherichia genus were isolated from indoor air of toilets. While, Malgorzata GS et al. [13] recorded that the bacterial species from Micrococcus and Bacillus genera were predominant indoors,(Micrococcus luteus, Micrococcus spp, Staphylococcus warneri, Bacillus pumilus and Bacillus cereus) strains present in the air were also isolated in settled dust samples from the ducts of the AC systems. According to some previous studies the microbiological quality of indoor air is highly influenced by the microbiological composition of outdoor air, which itself very much influenced by environment, season, the weather and even daytime [9,37]. During this study, the cases observed of the most common disease in the investigated schools between teachers and students varied between acute disease (sore throat, stomach ache, cold, fever, tonsillitis, flu cough, headache and dizziness) and chronic disease (asthma, anaemia, urinary tract infection, rheumatoid, eczema, chest allergy) (Table 2). It was observed that the diseases were common when the student's age was between (11-16) years but, the diseases were fewer when the teacher's age was between (29-40). Results of this study confirm the considerable microbial contamination of most investigated school settings. It should be emphasized that a real hazard of such pollution levels may be more serious because there are suggestions that in some cases the level of total bacteria may be even up to 5-times higher than the number of cultural bacteria determined. It's clear that high contamination of indoor air at schools poses a serious problem both from the point of view of health protection and environmental engineering [38]. Exposure to these microorganisms may cause allergies, respiratory and immunotoxic diseases [4]. But, considered that a great number of those microorganisms are emitted into the air during talking, coughing, sneezing or peeling of the epidermis. Also, lack of cleaning and checking out of the heating, ventilation and air conditioning systems (HVAC) may allow microbial growth, which causes rhinitis, bronchitis, pharyngitis, pneumonia, conjunctivitis and keratitis in the users. While, Colleen Nelson [39] evaluating dangerous bacteria in some air conditioners may be making you sick it leads to Legionnaire's disease, or Pontiac fever. You can contract it by inhaling a water organism often found in air conditioners. Also, Teklu S et al. [40] states that hot temperatures and rain are turning air conditioners into potential breeding grounds for bacteria. But they still suggest cleaning the filters of your air conditioners every few months. With the cooler months approaching he says it becomes even more important to make sure your air conditioner is clean (Table 3).

Results obtained in this study showed that the bacterial species varied in their susceptibility to all the antimicrobials used. Majority of them showed resistance to one or more of antimicrobials compounds. It was observed that Ciprofloxacin, was the most effective antibiotic to all bacterial species used during this study. The same instance was evident in a study conducted by Mohammedaman M et al. [41] who reported that all gram-negative bacteria isolates were susceptible to Ciprofloxacin. In the present investigation, Cefuroxime, Cefotaxime, Clindamycin and +Doxycycline were effective to all bacterial species except Pseudomonas stutzeri, which showed high resistance to

| S    | Nitrofurantoin | Fusidic Acid | Mupirocin | Rifampicin | Trimethoprim-
|------|----------------|--------------|-----------|------------|----------------|
| =16< | S              | S            | S         | S          | S              |
| =0.5<| S              | S            | S         | S          | S              |
| =2<  | I              | I            | I         | I          | I              |
| =0.5<| S              | S            | S         | S          | S              |
| =10< | S              | S            | S         | S          | S              |

**Table 4: Antimicrobial agent's susceptibility of isolated bacteria.**

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Nitrofurantoin (256) and also resistance to Celizolin and Cefoxitin (64), but it was sensitive to Trimethoprim/Sulfamethoxazole [40]. While, Mulu A et al. [42] stated that Gentamicin, Norfloxacin, Ciprofloxacin, Vancomycin and Amikacin were the most effective antibiotics to all bacterial isolates. In this study, Staphylococcus aureus was resistance to Benzylpenicillin, +Amoxicillin, +Ampicillin, +Cefixime, +Ceftazidime. This was consistent with study done elsewhere [43-47]. The same isolate was intermediate to Rifampicin and it was sensitive to Vancomycin beside 25 antibiotic compound as shown in Table 4. This study is in agreement with the work of Bibi S et al. [48], Shamsuzzaman A et al. [49], Gelaw A [50], Shriyan A et al. [51]. WHO reported that clinical Staphylococci are 100% sensitive to Vancomycin. Also, in this study, Ralstonia mannitolytica was sensitive to1 antibiotics compound Table 4. However, K. kistinae and F. tularensis were sensitive to 9 antibiotics and were resistant to Azithromycin, while these two species recorded the least resistance to Trimethroprim-Sulfame. This study was comparable with the previous studies done by Chalachew G et al. [24] and Sneath PHA et al. [25] (Table 4).

Conclusions

The bacterial profile of indoor air samples showed that Pseudomonas stutzeri, Ralstonia mannitolytica and Staphylococcus aureus, were isolated from the special schools, also Pseudomonas stutzeri was isolated from the air conditioners. But, Kocuria kristinae, Staphylococcus aureus and Francisella tularensis were isolated only from the indoor air of public schools. Among the bacterial pathogens present in the tested classrooms there were Francsella tularensis, the causative agent of tularemia. It was found that the highest bacterial concentration in heavily populated in workplaces. Also, the highest level of bacterial contamination was detected in the class rooms during lessons. Relatively high concentrations of bacteria was observed in the public schools (where overcrowded classrooms). Lack of cleaning and checking out of the heating, ventilation and air conditioning systems (HVAC) may allow microbial growth, which causes several diseases in the users. Cleaning the filters of air conditioners every few months. With the cooler months approaching it becomes even more important to make sure that air conditioner is clean. The study revealed that there was alarmingly high level of antimicrobial resistance among bacteria isolated from 252 samples of indoor air and air conditioners of 25 schools (Middle Schools) of the total isolates were resistant to 1 or more antibiotics. It was observed that Ciprofloxacin was the most effective antibiotic to all bacterial species used during this study.

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