How to Decipher a Microbial Puzzle on Microbial Control: Hands On

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Abstract

Antibiotics are among the most commonly prescribed drugs, but they are often misused,contributing to bacterial resistance, being an important public health problem, with clinical/economic impacts.

Aiming to contribute to convey information to students on Microbiology/antibiotic use, we developeddifferent teaching activities, with 6th and 9th grade students. To assessthe impact of these activities and student learning, we applied a pre-validated questionnaire before and after completion of the activities (pre- and post-test). We observed that most students had preconceived ideas that antibiotics were effective against viruses, however, after the implemented activities, the performance of students in the post-test improved significantly for both student grades. Regarding the use of antibiotics in specific diseases, students showed lack of knowledge concerning the treatment of cold, flu and tuberculosis, with some improvement in the post-test. Concerning antibiotic use, the initial knowledge of the 9th grade students was better than the 6th grade, nevertheless the highest improvement in knowledge was observed for the 6th grade students.

Our results support the use of teaching activities on Microbiology/antibiotic use that promote the development of critical thinking, analyze and solve problems. Our results support the need to educate students/communities on the proper use of antibiotics.

KEYWORDS: Experimental activities; Antibiotics; Microbiology/Molecular Biology, Public Health

Introduction

Antibiotics are among the most commonly prescribed drugs, but they are often misused (Chambers2006,World Health Organization: Report on Infectious Diseases 2000); their unnecessary use contributes to progressive loss of bacterial sensitivity (Centers for Disease Control and Prevention (CDC) 2012), being an important public health problem, with clinical and economic impacts (Maragakis, Perencevich and Cosgrove2008. Factors related to the misuse of antibiotics include self-medication, sharing with others, not completing the treatment, as well as saving antibiotics for later use (World Health Organization: Report on Infectious Diseases 2000,Grigoryan, Burgerhof,Haaijer-Ruskamp, Degener,Deschepper, Monnet, Di Matteo,Scicluna, Bara, Lundborg,Birkin, and SAR group 2007).

In Portugal, as in most countries, antibiotics require prescription for supply. However, several studies have shown that users from other countries can obtain them without prescription (Pechere2001).A studyrevealed that 60% of Europeans do not know that antibiotics are ineffective against viruses (Eurobarometer2001), so the lack of information in this field translates into high rates of consumption.A more recent study confirms that the expectation of antibiotic efficacy for common cold symptoms is very high (47.3%) in the general population (Oh, Mohamed,Mahmoud, Syed, Asrul, Ahmed 2011).A study performed by Jones and Rua, with 5th, 8th and 11th grade students also revealed a common misconception held by students: “the belief that “antibiotics can cure viral infections” and large gaps in the function of antibiotics (Jones&Rua 2008).Thus, information of the population on this field is mandatory. Educational campaigns have had a positive effect on knowledge/reduction in antibiotic prescription in different countries (Kiang,Kieke, Como-Sabetti,Lynfield,Besser, Belongia2005).

According to the above-described, health promotion interventions are decisive. Schools, are therefore the ideal place to work, because they give accesstoa large population of students and parents, and are respected institutions within the Communities.Similarly to other countries, studies performed in Portuguese schools showed lack of knowledge on antibiotics and their correct use, which could result to insufficient formal education in this issue (Azevedo,Pinheiro, Yaphe, Baltazar2009). From the analysis of the Portuguese Natural Sciences curriculum, it can be seen that very little time is spent in the area of Microbiology, and the contents on this field are not developed in depth in subsequent years.

Given the urgent need to convey important information to students about the practical aspects in the field of Microbiology/antibiotic use, we decided to develop teaching activitiesfor6th and 9th grade students. To validate these activities and assess student learning, students were asked to answer a pre-validated questionnaire before and after completion of laboratory activities (pre-test and post-test). The specific research questions to answer were: (a) To what extent these experimental teaching activities influence the acquisition of scientific knowledge? (b) To what extent the educational level of students (6thgrade/9thgrade) affects the acquisition of scientific knowledge, after the implementation of these activities?

Materials and methods

Sample population

This study wasincluded in Prize IlídioPinho Foundation for the program "Science in School" and was undertaken at the School EB 2,3 D. Maria II,V.N. Famalicão, districtof Braga. This is an unprecedented work, first held in Portugal. For this work we used a convenience sample,which included 26 students from 9th grade and 18 students of 6th grade. The population of the 9th grade corresponds of 31% of the total number of students of the 9th grade and 18% of the 6thgrade. This study was approved by the School Board after hearing the Pedagogic Council.

Materials and Procedures

The results were obtained through the application of a pre-validatedquestionnaire used in a previous study (Azevedo, Pinheiro, Yaphe, Baltazar2009) before and after the implementation of a series of experimental activities (pre- and post-test). The time between the implementation of the activity and application of the post-test was 2 months. The questionnaire contains 7 questions. The first 2 questions include5 items related toassociations between organisms/diseases and antibiotics/organisms. The remaining questionsare related to the correct use of antibiotics. The questionnaire was resolved during the course of a regular class with a time limit of 30 minutes. Participation was anonymous and voluntary.

In a second phase, through a presentation (powerpoint), followed by discussion, the contents above were explored with students in a regular class during 90 min. The first part of this presentation contained basic information on microorganisms such virus, bacteria, fungi and protozoa. The second part was related to diseases with focus on the discrimination of drugs against the different types of microorganisms. Relevance was given to the treatment of diseases caused by bacteria, giving particular attention to tuberculosis. In this perspective, the presentation centered the attention on antibiotics, such as: 1) story of antibiotic discovery, 2) effectiveness of antibiotics against bacteria, 3) correct use of antibiotics and, 4) the recent problem of antibiotic resistance.

Later, the students learned to make microscopic preparations (fresh exam) of different microbial groups (bacteria, unicellular and filamentous fungi). Next, theyobserved the slides that they prepared: *Lactobacillus casei* and *Escherichia coli* (bacteria) *Saccharomyces cerevisiae*and *Schizosaccharomycespombe*(unicellular fungi) and *Heliscussubmersus* (filamentous fungus).The students illustrated scientifically their microscopic observations usingthe appropriate magnifications. In this step, the students were alerted to the different dimensions of the microorganisms as well as to their morphology/structure.

In the next phase, students prepared Muller Hinton Agar culture medium to perform an antibiogram. At this stage they also learned to sterilize laboratory equipment (Erlenmeyer flasks, pipettes, Petri dishes and test tubes). Before starting this step, students were given an experimental protocol with all the procedures to accomplish such aseptic techniques; preparing the material for sterilization; sterilization by dry heat and moist heat.

After completionof the sterilization process, the students distributed aseptically the culture medium in Petri dishes and to later performtheantibiogramby inoculating the above-mentioned microbial strains. After,they placed three disks impregnated with antibiotics (tetracycline, penicillin, streptomycin) in each of the plates containing the microbial strains. Several controls were used including: a Petri dish containing only the culture medium, and Petri dishes containing the culture medium plus each of the test strains in the absence of antibiotics.

The next phase "Identification of microbial strains with different clinical interest by restriction of genomic DNA" was applied only to students from the 9th grade because of its complexity, and was divided into 2 stages. The first stepconsisted of a brief theoretical discussion on basic concepts of Microbiology/Molecular Biology indispensable for understanding the practical activity to perform, and exploitation of a virtual lab (Learn.Genetics™2011) that exemplified the tasks that students would have to reproduce.

In the second step, students have started performing experimental work, preparing an agarose gel, where samples of genomic DNA (extracted in advance) from different microbial species were subjected to an electric field. The genomic DNA was previously treated with a restriction enzyme. Once completed the electrophoresis, the different patterns of restriction from the different microorganisms were visualized under ultraviolet light. This technique allows the correct identification of the microorganisms under study.

The activities comprised the experimental implementation of the activities described above, in 13 experimental classes (45 min each). The protocols were previously analyzed and discussed with the students prior to their execution. After these experimental activities, students performed the post-test in order to investigate the evolution of knowledge.

As we consider the dissemination of this work/ results of extreme importance, various means of dissemination were used: the Website of the School; local newspapers and a lecture held for the whole School Community (students, staff of the school and parents).

Data Analysis

Data obtained in the pre- and post-test were analysed using the SPSS software for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA). Associations between variables were tested with Pearson's Chi-square (χ^2) with significance set at $p<0.05$.

Results

Experimental activities

The experimental results refer to the following experiments 1- Correct mounting of the slides for microscopic observation (Fig. 1); 2 Preparation of culture media/sterilization of material (Fig. 2A and 2B); 3-Execution of an antibiogram (Fig.3A and 3B) 4 - Identification of different microbial strains by DNA electrophoresis on an agarose gel (Fig. 4A, 4B and 4C).

These experimental results can be classified into qualitative,including the preparation of the slidesand culture media and sterilization of medium/ material; and quantitative, including the antibiogram and species identification of microbial strains by genomic DNA restriction.

Antibiogram results

In the case of *S.cerevisiae*, *S.pombe*, and *H.submersus*, the antibiotics used did not inhibit the growth of these microorganisms, since they grew on the entire surface of the Petri dish. This result demonstrates that the spectrum of antibiotics do not include fungi.

Regarding *E. coli*, the antibiotics used inhibited bacterial growth in an identical manner, demonstrating the activity of antibiotics againstbacteria (Fig. 5). Regarding*L.casei*, streptomycin was effective to inhibit its growth but penicillin was not.

Controls

1-Petri dishes with culture medium only. The result was the expected, since after 3 days of incubation the dish remained sterile, demonstrating that the culture medium was properly sterilized.2-Petri dishes with culture medium plus test strain. As expected, all microbial strains grew after 3 days of incubation.

Identification of microbial strains by genomic DNA restriction

This experiment was intended to demonstrate that it is possible to identify microbial strains by DNA electrophoresis. Students prepared an agarose gel with ethidium bromide as a marker of nucleic acids, where samples of genomic DNA (previously extracted) from different microbial strains were subjected to an electric field. Students were closely supervised in this procedure due to the dangers of ethidium bromide manipulation.

The genomic DNA was previously treated with a restriction enzyme. Students prepared the samples by adding loading buffer to DNA. ADNA molecular weight marker was included. After the electrophoresis, the restriction profiles of the different microorganisms were visualized under ultraviolet light (Fig. 6). Analysis of the gel patternsdemonstrated that all strains used were different.

Pre- and post-test results

The response rate of the applied questionnaire was 100% among the students asked to participate, 26 from 9th grade and 18 students of the 6th grade, asked to participate.

Students' knowledge regarding the use of antibiotics against microorganisms were initially evaluated by applying a pre-test, Question 1. In this area, the students revealed some difficulties, since only 38.9% and 7.7% from the 6th grade and 9th grade,respectively, said that antibiotics were not effective against viruses (Table 1 and 2). Regarding the effectiveness of antibiotics against bacteria, 61.1% and 80.8% of the students from the 6th grade and 9th grade, respectively, answered correctly (Table 1 and 2, respectively). Comparing with the post-test, there was a significant increase in the number of correct answers in the 6th grade concerning the use of antibiotics against viruses, bacteria and fungi (p<0.005, p=0.041, p=0.018, respectively, Table 1). Regarding the antibiotic activity against other organisms, there was no significant increase in the number of correct answers. In the9th grade students,there was only a significant increase in the number of correct answers inQuestion 1, regarding the use of antibiotics against viruses (p<0.001) and parasites (p=0.009) (Table 2), comparing pre- and post-test.

Regarding the use of antibiotics in the treatment of specific diseases such as cold, flu, tuberculosis, AIDS and diabetes (Question 2),the 6th grade students showed generalized lack of informationin the pre-test (Table 1), except for AIDS treatment in which the number of correct responses was 72.2%. However, the performance of the students improved significantly in the post-test with regard to the inadequate use of antibiotics to treat cold (p=0.041), flu (p=0.041) and diabetes (p=0.003) (Table 1). In the pre-test, the level of information of the 9th grade students was similar to the 6th grade, except for the question of the treatment of diabetes, in which 84.6% of students answered correctly. However, comparing with the post-test, there were significant improvements in the question of the use of antibiotics in treatment offlu (p=0.011), and AIDS (p=0.021) (Table 2). The initial similar results of 6th and 9th grade students may be related to the fact that these contents are not matter of study in the 7th and 8th grade.

Regarding the use of antibiotics (Questions 3 to 7), students of the 6th and 9th grade revealed similar difficulties in the pre-test (Table 1 and 2, respectively), mainly on Question 4 "Antibiotics may be taken at different time intervals if the daily doses are respected". Only 38.9% and 42.3% of the students from the 6th and 9th grade,respectively, answered correctly. In the post-test, the performance of the 6th and 9th grade students improved for questions related to antibiotic use (Table 1 and 2, respectively). However, for both years of schooling there were only significant improvements in Question, with p=0.041 for 6thgrade and p=0.025 for 9thgrade (Table 1 and 2, respectively).

Discussion

This research assessed the effectiveness of a set of practical activities implemented in the Portuguese Basic School D. Maria II, V.N. Famalicão to students from 6th and 9th grade, aimingtoimprove the knowledgeon use and misuse of antibiotics.We used a convenience sample of students in order to allow a rapid data collection in a short time, with limited resources. Information on the socio-economic and intellectual level was not collected.

Overall, the teachers involved in the activity had the perception that the students felt that the experience was very constructive and motivating,mainly due to the experimental activities; students were allowed, whenever possible, to work independently but with previous guidance, having enough time to perform the proposed tasks. The classroom and laboratory activities were proposed to illustrate the nature of science and introduce students to the thinking process and methods in science, such as formulating and testing hypotheses, distinguishing between observations and deductions, controlling variables and developing experiments. However, the most interestingpart of the laboratory work are "surprises" that may occur, which are the best incentives for learning, since they allow to experience the adventure of the scientific process.

One of the key roles of the teacher should be to help students making connections between what they are seeing and its meaning with real scientific validation. The purpose of these experimental activities/discussions was to introduce new concepts, reinforce concepts covered in lectures, apply these concepts to new situations and motivate students for experimental work using all the steps of the scientific method.

Regarding the results obtained in the pre- and post-tests, with respect toQuestion 1, on the effectiveness of antibiotics in treating diseases caused by various organisms, we observed that most students had preconceived ideas that antibiotics were effective against viruses, however, after the implemented activities, including the presentation on "Microbial World" developed by the authors of this study (FB and MMA), the performance of students in the post-test improved significantly for both 6th and 9th gradestudents. These results corroborate the ones of Azevedo and colleagues (Azevedo, Pinheiro, Yaphe, Baltazar2009), who showed that there are marked deficiencies in Portuguese students' knowledge regarding antibiotics and their correct use, suggesting the introduction of Curricular Unit of Microbiology in 9th and 12th grade in all curricular areas.Cebotarenco(Cebotarenco1999)reported that in times of high incidence of virus infections, half of these infectious diseases are treated with antibiotics byself-medication. Some authors suggested that children need to be educated on correct antibiotic use(Standing Medical Advisory Committee Sub-Group on Antimicrobial Resistance 1998) and some Educational Programs have been developed (Cebotarenco&Bush 2008, Lecky, McNulty,Touboul,Herotova,Beneš et al. 2010).The experimental activities were performed with bacteria and fungi, but not with virus since they are difficult to handle in the laboratory and have higher intrinsic dangerousness, however this issue was exploited in the powerpoint presentation. We believe that these laboratory experiments contributed to the improvement of the knowledge of the students on this matter.

Regarding Question 2, use of antibiotics in the treatment of specific diseases, the students, still show some lack of knowledgein the post-test, in particular with regard to the treatment of cold, flu and tuberculosis. In our opinion, the implemented activities helped students to make the connectionsbetween organisms and antibiotic, but they will need to further strengthen the connection between organisms and disease. Changing these preconceived ideas is extremely important since the population, in general, continues to believe that antibiotics are effective in the treatment of colds and flu. Recent studies have shown that mothers often influence medical decisions regarding the prescription of antibiotics (Cebotarenco&Bush2008). In this context, pediatricians are often pressured to prescribe antibiotics for children with viral infections (Mangione-Smith, McGlynn, Elliott 1999, Palmer&Bauchner1997).

Concerning the questions related to the use of antibiotics (Questions 3-7), comparing to the 6th grade, the initial knowledge (pre-test) of the 9th grade students was quite satisfactory, with most correct answers above 80%, nevertheless the highest improvement in knowledge was observed for the 6th grade students. The latter observation allows to conclude that the activities were effective. The biggest changes were observed for Question 4 for both grades, since the pre-test results were quite low (around 40 %). This may be due to the emphasis given to this matter during the presentation.

The increasing worldwide prevalence of bacterial resistance to antibiotics, partly due to the widespread and incorrect use, constitutes a threat to public health. Thus, increasing the public awareness of the problem and education of the general population and retailers on the proper use of antibiotics can help to slow this trend (Cebotarenco&Bush, 2008, Mangione-Smith,McGlynn, Elliott 1999,Wenzel, Edmond, 2000,Wachter, Joshi,Rimal 1998), preventing the emergence of resistant strains that induces considerable morbidity and mortality. With this aim, we used various means for the divulgation of this Project/results obtained: Internet Site of the School; Local newspapers and a Lecture performed by one of the authors of this manuscript (MMA) directed to the whole school community (students, teachers, staff of the school and parents). This lecture sought to sensitize the educational community to the problem of antibiotic resistance.

The post-test was administered approximately 2 months after completion of the experimental activities, however, it would be important to re-apply it after a longer period to infer on the long term retention of knowledge. The next step could be to try other methodologies, including e-learning, and compare them with the experimental methodologies used in this study to infer the best way to convey this knowledge.

Concludingour results support the use of appropriate methodologies, more specifically activities of teaching Microbiology in the laboratory, that promote the development of critical thinking, the ability to interpret, analyze and solve new problems. The results also support the need to educate students and communities on the proper use of antibiotics. In conclusion, the laboratory work seems to be valuable in Science Education, according to Tao Te King saying "I hear and I forget, I see and I remember, I do and I understand”.

Conflict of Interest

The authors declare no conflicts of interest.

References

Azevedo, M.-M., Pinheiro, C., Yaphe,J., Baltazar, F.(2009). Portuguese students' knowledge of antibiotics: a cross-sectional study of basic, secondary and university students in Braga. BMC Public Health, vol.9, no. 359.

Cebotarenco, N. (1999).Children's knowledge about antibiotics.BuletinulInstitutuluiNational De Farmacie, vol. 3, pp.2-14.

Cebotarenco, N. and Bush, P. J. (2008).Reducing Antibiotics for Colds and Flu: A Student Taught Program.Health Education Research,vol.23, pp.146-157.

Chambers,H. F.(2006).General principles of antimicrobial therapy.In Brunton L, Parker K, Blumenthal D & Buxton I (Org.).Goodman& Gilman's The Pharmacological Basis of Therapeutics (pp. 1095-1110). McGraw Hill, NewYork.

Centers for Disease Control and Prevention (CDC) Available online athttp://www.cdc.gov/Features/GetSmart/.Assessed on 15 January 2012.

Eurobarometer2001. Available online at: http://ec.europa.eu/research/press/2001/pr0612en-report.pdf. Assessed on February 15 2013.

Grigoryan, L., Burgerhof J. G., Haaijer-Ruskamp, F. M., Degener, J. E., Deschepper, R., Monnet, D. L., Di Matteo, A., Scicluna, E. A., Bara, A. C., Lundborg, C. S., Birkin, J. and SAR group. (2007). Is self-medication with antibioticsin Europe driven by prescribed use?Journal of Antimicrobial Chemotherapy, no. 59, pp.152-156.

Jones, M.G., Rua M.J. (2008).Conceptual Representations of Flu and Microbial Illness Held by Students, Teachers, and Medical Professionals.*School Science and Mathematics*, vol.108, no. 6, pp. 263–278.

Kiang, K.M., Kieke, B.A., Como-Sabetti, K., Lynfield, R., Besser, R.E., Belongia, E.A. (2005).Clinician knowledge and beliefs after statewide program to promote appropriate antimicrobial drug use.Emerging Infectious Diseases, vol.11, pp. 904-911.

Learn.Genetics™, Genetic Science Learning Center, The University of Utah.Available at http://learn.genetics.utah.edu/. Assessed in September 2011.

Lecky, D.M, McNulty, C.A.M., Touboul, P., Herotova, T.K., Beneš, J., et al. (2010).Evaluation of e-Bug, an educational pack, teaching about prudent antibiotic use and hygiene, in the Czech Republic, France and England.Journal of Antimicrobial Chemotherapy, vol.65, pp. 2674–2684.

Mangione-Smith, R., McGlynn, E. A., Elliott, M. N. (1999).The relationship between perceived parental expectations and paediatrician antimicrobial prescribing behavior.*Pediatrics*, vol. 103, pp.711-718.

Maragakis, L. L., Perencevich, E. N. and Cosgrove, S. E. (2008).Clinical and economic burden of antimicrobial resistance.Expert Review of Anti-Infective *Therapy*.6, pp.751-763.

Oh, A.L., Mohamed, A.H., Mahmoud S.A., Syed, A.S.S., Asrul, A.S., Ahmed, A. (2011). Public knowledge and attitudes towards antibiotic usage: a cross-sectional study among the general public in the state of Penang, Malaysia. The Journal of Infection in Developing Countries, vol. 5, no.5, pp. 338-347.

Palmer, D.A., Bauchner, H. (1997). Parents' and Physicians' views on antibiotics.Pediatrics, 99 (6), E6.

Pechere, J. C. (2001). Patient's interviews and misuse of antibiotics.Clinical Infectious Diseases.33(Suppl 3), pp. S170-S173.

Standing Medical Advisory Committee Sub-Group on Antimicrobial Resistance 1998.The Path of Least Resistance.Available at http://www.advisorybodies.doh.gov.uk/smac1.htm (Assessed in September 2012).

Wachter, D. A., Joshi, M. P., Rimal, B. (1998). Antibiotic dispensing by drug retailers in Kathmandu, Nepal.Tropical Medicine and International Health.vol.4, pp. 782-78.

Wenzel, R. P., Edmond, M. B. (2000). Managing antibiotic resistance.New England Journal of Medicine.vol.343, pp. 1961-1963.

World Health Organization: Report on Infectious Diseases.Overcoming antimicrobial resistance.Available online at http://www.who.int/infectious-disease-report/2000/index.html. Geneva: World Health Organization 2000. Assessed on January 3 2011.

Table 1- Correct answers of6th grade students (%) in the pre-test (pre-) and post-test (post-).

6th grade students	Questions	% Correct answers			*P*
		Pre-	Post	Δ (Post-Pre)	
Question 1 Antibiotics/microrganisms Antibiotics are effective against:	Virus	38.9	88.	50.0	<0.00
	Bacteria	61.1	94.4	33.4	0.041
	Fungi	55.6	94.4	38.8	0.018
	Parasites	72.2	83.	11.1	0.691
	Insects	61.1	88.9	27.8	0.121
Question 2 Antibiotics/diseases	Cold	38.9	77.	38.9	0.041
	Flu	22.2	61.	38.9	0.041
	Tuberculosis	33.3	55.	22.3	0.180
	AIDS	72.2	83.	11.1	0.691
	Diabetes	44.4	94.4	50.0	0.003
Questions 3-7 Antibiotic use	3-Antibiotics do not interact with alcohol	94.4	100	5.6	1
	4-Antibiotics can be taken at different time intervals, if the daily doses	38.9	77.	38.9	0.041
	5-Antibiotic treatment should be stopped as soon as the patients feel	66.7	77.	11.1	0.711
	6-Antibiotics could be shared with other people if the symptoms are similar	72.2	83.3	11.1	0.691
	7-The incorrect use of antibiotics can lead to development of resistant bacteria	66.7	88.9	22.2	0.228

Table2- Correct answers of 9th grade students (%) in the pre-test (pre-) and post-test (post-).

9th grade students	Questions	% Correctanswers			*P*
		Pre-	Pos	Δ (Post-Pre)	
Question 1 Antibiotics/microrganisms Antibiotics are effective against:	Virus	7.7	76.	69.2	<0.00
	Bacteria	80.8	8.5	7.7	0.703
	Fungi	65.4	73.1	7.7	0.548
	Parasites	57.7	92.	34.6	0.009
	Insects	65.4	84.6	19.2	0.199
Question 2 Antibiotics/diseases	Cold	53.8	57.	3.9	0.780
	Flu	23.1	57.	36.4	0.011
	Tuberculosis	26.9	46.	19.3	0.150
	AIDS	73.1	96.	23.1	0.021
	Diabetes	84.6	96.2	11.6	0.350
Questions 3-7 Antibiotic use	3-Antibiotics do not interact with alcohol	96.2	10	3.8	1
	4-Antibiotics can be taken at different time intervals, if the daily	42.3	73.	30.8	0.025
	5-Antibiotic treatment should be stopped as soon as the patients feel	84.6	96.	11.6	0.350
	6-Antibiotics could be shared with other people if the symptoms are similar	88.5	100	11.5	0.235
	7-The incorrect use of antibiotics can lead to development of resistant bacteria	88.5	100	11.5	0.235

Figure 1.Observation of microorganisms under the microscope.

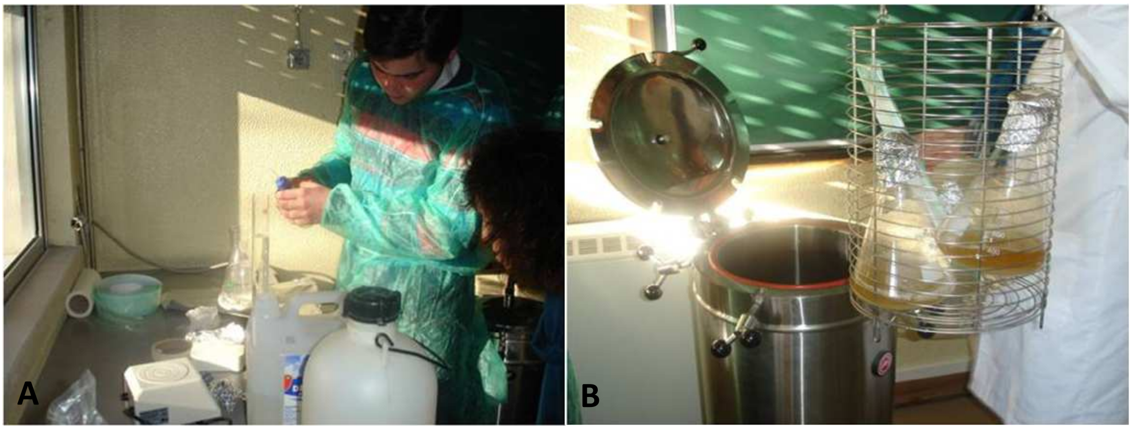

Figure 2. A- Preparation of culture medium; B- Sterilization of material in an autoclave.

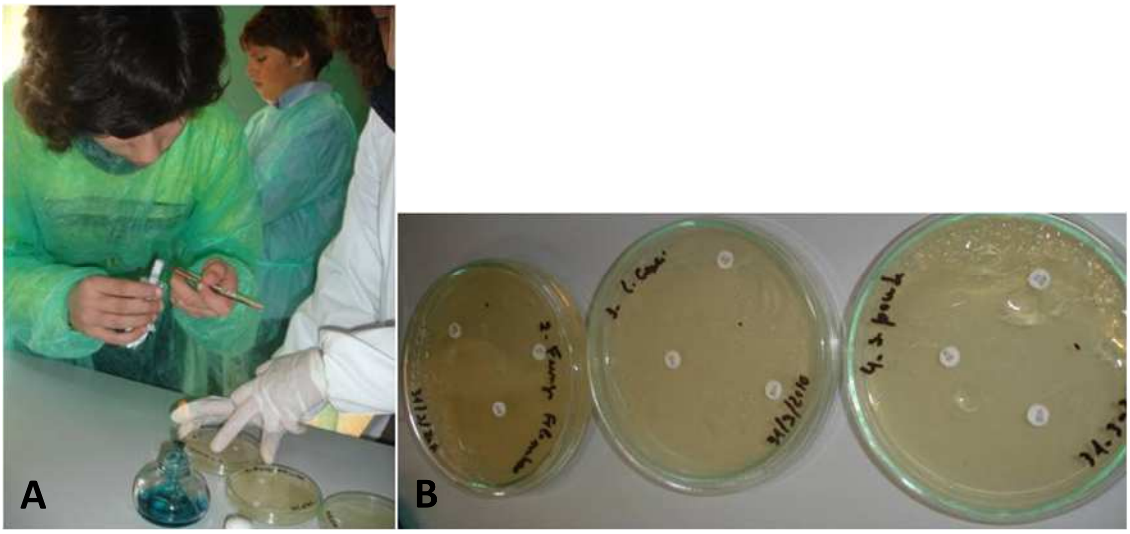

Figure 3. A- Preparation of the antibiogram; B- Petri dishes containing the antibiotic disks.

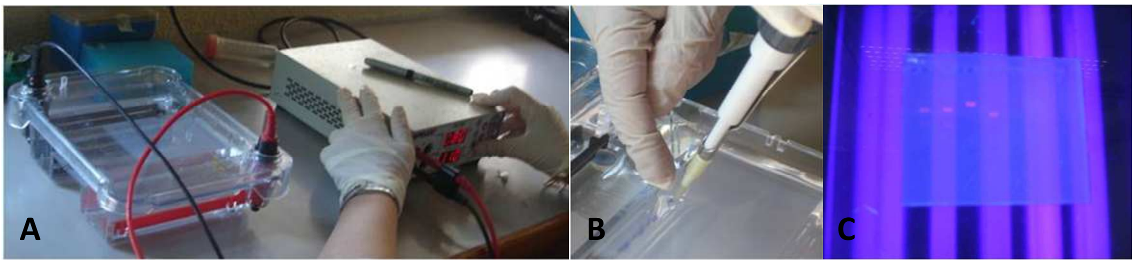

Figure 4. A- Eletrophoresis equipment; B- DNA sample loading into anagarose gel; C-DNA restriction profiles of the different microorganisms visualized under UV light.

Figure 5.Antibiogram results, showing the growth inhibition areas of the bacteria.

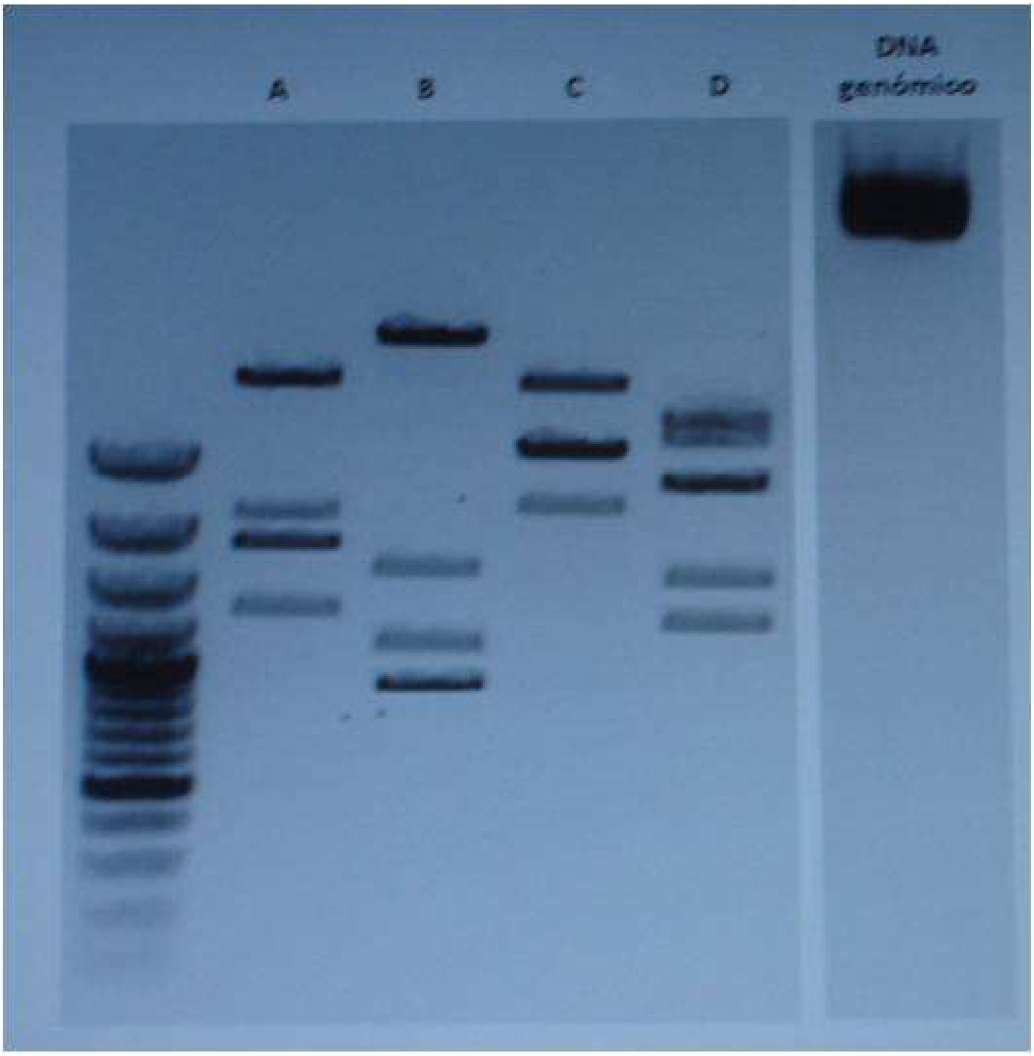

Figure 6. DNA restriction profiles of the different microorganisms visualized under UV light.