

A Microbiological Web-Based Database For Bacteria Resistant To Antibiotics Used In Aquaculture: A User-Friendly Structure.

S.Bertone^{1,2}, C.Ruggiero³, F.Raffo³ and M.Giacomini³

¹R.I.L.A.B. srl, Genova, Italy.

²DISCAT, Microbiology Section, University of Genova, Italy.

³Dept. of Communication Computer and System Science, University of Genova, Italy.

ABSTRACT

This web-based data base (DB) application has been designed in order to collect data coming from microbiological samples from aquaculture systems. The European founded project ASIARESIST (Hazard analysis of antibiotic resistance associated with Asian aquacultural environments - ICA4-CT-2001-10028 - INCO-DEV Program) aims to study bacteria resistant to antibiotics used in aquaculture coming from fish water and sediment samples of Asian aquaculture farms (Malaysia, Thailand and Vietnam). The aim of the project is to investigate the possibility of antibiotic resistance transfer between bacteria coming from aquaculture samples and bacteria such as *Escherichia coli* commonly present in the human intestine.

INTRODUCTION

This web-based data base (DB) application has been designed in order to collect data coming from microbiological samples from aquaculture systems. The European founded project ASIARESIST (Hazard analysis of antibiotic resistance associated with Asian aquacultural environments - ICA4-CT-2001-10028 - INCO-DEV Program) aims to study bacteria resistant to antibiotics used in aquaculture coming from Asian aquaculture farms (Malaysia, Thailand and Vietnam). The problem arises from the large use of various antibiotics in aquaculture, both for treatment and for prevention of diseases in aquatic species. While the use of these antibiotics are important for many farmers in order to maintain their activities, since effective vaccines for aquacultured species are not developed yet for all important pathogens, their use presents potential problems for the end user. Fish and crustaceans are usually farmed at high densities and the antibiotic administration through food or water leads to high antibiotic concentrations in the aquatic environment in the farm proximity, causing high probability of antibiotic resistance development in the residential microflora. Although most of the environmental bacteria that occur in the proximity of aquaculture farms are non pathogenic for animals and humans, when they develop resistances to the various antibiotics used in the farms, it can become a risk for the population. In fact, due to the capability of bacteria in natural environments to transfer genetic information among themselves, an important way of genetic renewal in Prokaryotics, the antibiotic resistances could find their way to human pathogens occasionally present in such environments. This causes a significant risk to the end user (Midtvedt and Lingaas, 1992; Sandaa et al., 1992). Thus, this project has the aim to investigate the antibiotic resistances that may occur in aquaculture systems in South East Asia, to study the possibility of genetic transfer of such resistance between bacteria, and to asses the risks for the end users.

Antibiotic usage in aquaculture is legitimate as regards some type of antibiotics. Chloramphenicol (CHL) is an example of antibiotic not permitted, due to its high toxicity for the humans. The recent evidence of its illegal use in aquacultures of shrimp and prawns in producing countries of Asia, has caused the rejection of Asian aquaculture products by the

European Union. Starting from this point, CHL was selected as the screening antibiotic, i.e. the antibiotic against which the resistant bacteria are selected for the successive studies.

METHODS

The internet is the fastest growing tool and arguably the most popular method of accessing public domain information. When choosing an architecture for a data storing and retrieval application, the designer has to face a number of issues that really guide or focus the development process. Moreover, since the DB aims to collect all data coming from the project in a standard and clear format, it was developed to be user friendly with all partners, showing interdisciplinary and user-friendliness through visual animations, mimicking the real experiments.

The Web application was implemented using ASP.NET, a set of technologies in the Microsoft .NET Framework.

ASP.NET (MacDonald 2002) pages contain server-side logic written in both C# (Petzold 2002) and Visual Basic.NET (Balena, 2002) languages. This application realizes a front-end for the DB developed using MS Access 2000.

These pages were integrated at the client-side using Macromedia Flash MX, an excellent tool typically used on the Internet for developing highly visual interactive animations.

Applications that store sensitive information need to be protected from malicious attacks and from competitors attempting to steal information or intellectual property. To obtain a protected Intranet it was planned to authenticate users using IIS (Internet Information Service) Authentication coupled with the Forms Authentication implemented in ASP.NET. Using Forms Authentication causes unauthenticated requests to be redirected to a specified ASP.NET page using client side redirection. The user can then supply logon credentials, and post them back to the server. If the application authenticates the request (using application-specific logic), ASP.NET issues a cookie that contains the credentials or a key for reacquiring the client identity.

RESULTS

The first page of the web based DB is a login page asking for a USER ID and a PASSWORD. Since the access security is one of the most important features when considering on-line DB, at the release the User database contains only the responsible of each country, so that they are the only authorised persons to access the database. They can authorise other persons, but only for manipulating data related to his/her country. The DB contains several sections, in the data insertion ones, the animations resemble the equipment used in laboratory experiments, in order to assure the maximum user-friendliness for the operator. The main page contains four sections.

The Main Page is divided in 4 sections marked in different colours. These sections are not all open to all partners, for security reasons only the partner entitled to produce that kind of data can enter the specific section of the database.

The first section is open to all partners. It allows the user to modify its own data, password enclosed (modify user information), and allows the user to permit another collaborator/colleague to enter and use the data base (insert a new user).

The second section has two buttons: "Insert Site-Sample-Strain description" open only to Asian partners and "Confirm Strain", open only to the European partners.

The first button opens a new menu in which it is possible to:

1. add a new site: by clicking on this button the user can insert all info describing the new sampling site;
2. modify a site: it allows user to modify a site that has already been inserted.
3. insert all info that is required until the strain code is defined.

The strain code contains information about:

- Resistance/Non resistance to a certain concentration of chloramphenicol antibiotic
- Country of origin
- Sampling Site
- Fish farm pond
- Sampling period
- Sample type
- Colony number

The second button (Confirm Strain) allows the user (only European partners) to confirm specific strain codes in order to allow them to be selected in the following sections, based on the strain purity and suitability to be further studied.

The third section is open only to Asian partners. With the button “Antibiogram Test” it is possible to insert antibiotic resistance data for a chosen strain code. By clicking on the antibiotic short name, a table will appear where the user can insert the mm of the growth inhibition zone caused by that antibiotic. At the end, when all antibiotic data are inserted, by clicking on the button “view” it is possible to see the plate animation with inhibition zones, the figure mimicking the real laboratory test (Fig.1).

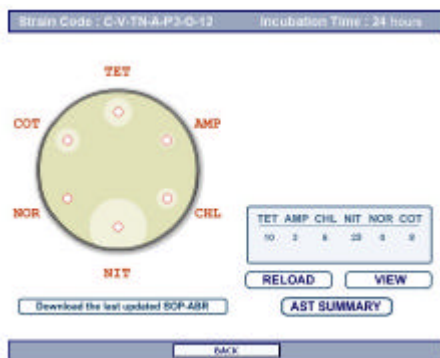


Figure 1. Antibiogram section

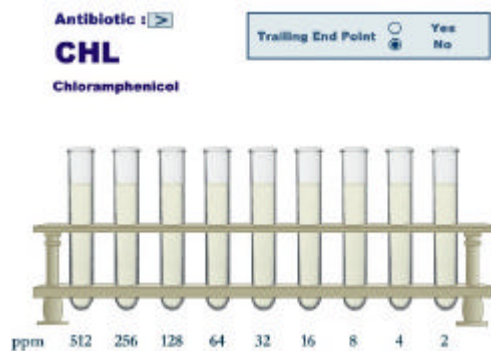


Figure 2. MIC section

At the end of this section, the “AST summary” button shows a table summarising the data inserted, resistant/susceptible interpretation according to the National Committee for Clinical Laboratory Standard guidelines (NCCLS, USA) is also reported.

With the button “MIC Test” it is possible to insert minimal inhibitory concentration data, for a chosen strain code. After selecting the antibiotic to insert data about, the user can insert the MIC by clicking on the corresponding tube. The image will change simulating the real results of the experiment (Fig.2).

The third section is open to all partners. It allows to Search for any strain inserted in the data base. All info inserted for that strain is summarised in corresponding tables.

DISCUSSION AND CONCLUSION

One of the main problems for the end user about the use of data base, software, PC, is very often, the lack of expertise and the lack of user-friendliness of the equipment available. This often leads to use the less possible the informatic instrumentation and/or to use it improperly. Because of the distance, and different culture and languages between partners, one of the main features of this Data Base is the user-friendliness. Particularly the Antibigram and MIC sections that are built up through animations mimicking the real experiments performed in the laboratory (same colours and shapes of the instrumentation are used and same visualisation of the results), allowing the user to insert data in a very simple way.

Two other topic points are data merging and data security. With actual technology these topics seem to be in competition, as for a manageable data merging protocol data input should be done on a shared platform from several data sources in some countries, but this makes the database less secure. The solutions described in this paper can represent a possible compromise solution of these topics.

The project is at its second year (over three years in all) and more sections of the data base will be developed, especially regarding the identification at genus and species level, and all the genetic data. Surely the characteristic of user-friendless of the first part will be maintained.

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