Drug Resistant *Streptococcus pneumoniae* (DRSP) in the Maltese Islands

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Abstract

The DRSP prevalence rate for the Maltese Islands was investigated. Consecutive samples were obtained, both from adults and children, from September 2000 through April 2002. Penicillin-intermediately-resistant isolates amounted to 27%, erythromycin-resistant isolates 31%, and clindamycin-resistant isolates 19%.

The oxacillin disk was found to be an effective screening method for the detection of penicillin resistance. An association was found in patients who had DRSP, as well as diabetes and/or cardiovascular disease. Finally, an investigation of the local antibiotic consumptions over the period 1997-2000, for the National Health Service was conducted. The highest consumption rates were obtained with co-amoxiclav, amoxicillin, erythromycin, cephalexin and ciprofloxacin.

The results obtained here call for more judicious use of antibiotics. In addition, the setting up of a local DRSP surveillance unit is mandatory. Moreover, the use of molecular techniques to investigate specific genes, such as *ermAM* and *mefE* associated with macrolide-resistance, should be introduced as part of investigational laboratory work.

Keywords

Drug-resistant *Streptococcus pneumoniae* (DRSP), antibiotic resistance, MLS_B (Macrolide, Lincosamide, StreptograminB) phenotype, *ermAM* gene, *mefE* gene, defined daily doses (DDD) /1000 inhabitants/day or DID.

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Introduction

The first report of acquired bacterial resistance, according to LP Garrod, dates back to 1937, when a Royal Navy surgeon-commander, TF Crean, observed a number of refractory cases of gonorrhoea in a group of patients treated with the sulphonamide, sulphanilamide (Prontosil®).[1] Selection of bacterial resistance started on a global scale in the early 1940s, when the first penicillins were introduced in clinical practice.[2]

Alexander Tomasz described the first penicillin-resistant pneumococcal isolate, (capsular type 4), isolated from the throat of a healthy 3-year-old boy in the village of Anguganak, New Guinea, with a population of 507, on April 15, 1969.[3,4] However, in 1967, Hansman and Bullen described a patient in Sydney, with hypogammaglobulinemia and bronchiectasis, in whom a strain of pneumococcus (Type 23) that was relatively insensitive to penicillin, was isolated from the sputum.[5,6]

Resistance to ß-lactams in the pneumococcus is mediated via an intrinsic alteration of high molecular weight penicillin-binding proteins (PBPs).[7] Cephalosporin resistance is due to alterations in PBP1a and 2x.[8]

Resistance mechanisms to macrolide, lincosamide (e.g. clindamycin) and streptograminB (MLS_B) antibiotics mainly involve:

a) Target site modification (encoded by *ermAM*), conferring broad cross-resistance to MLS_B antibiotics and/or
b) Active efflux pump alteration (encoded by *mefE*). This type of resistance is confined to structurally-related antibiotics only.[9]

Gram-positive bacteria are intrinsically less susceptible to fluoroquinolones than many Gram-negative bacteria. Consequentially, bacteria such as *Str. pneumoniae* typically require only one or sometimes two mutations in the genes encoding the target proteins to develop clinical resistance. Hence, this implies that fluoroquinolone resistance is more likely to occur.[10]

The most notoriously, widely spread epidemic clones of *Str. pneumoniae* include serotypes 23F, 14, 9V and 6B.[3,4]

A report by the United States, Centers for Disease Control (CDC), for the period April-June 1995, indicated that there was a great temporal and geographic variation in resistance patterns and this ranging from 2 to 30%. DRSP may spread quickly throughout a community and the prevalence rates may differ between children and adults.[11]

Table 1: *Breakdown of Results*

Alpha-Haemolytic Strains	Number of Isolates
Total number of strains tested	129
Viridans streptococci	48
Isolates which showed no growth	5
Pneumococci	76*
Total number of Resistant Pneumococci	37
Multi-Drug-Resistant Isolates	
Erythromycin + Clindamycin	18.9% (14/74)
Penicillin + Erythromycin + Clindamycin	8.1% (6/74)

**Two isolates were excluded as they were obtained from the same two patients, within 2 days of each other*

Reacher et al[12] conducted a study whereby resistant isolates obtained from blood and reported to the Communicable Disease Surveillance Centre, during the period 1990 to 1998, were investigated in England and Wales. It was observed that penicillin resistance in *Str. pneumoniae* was below 1% in 1990 and 1991, rising to 3.7% in 1996, reaching a value of 7.4% in 1997 and 3.6% in 1998. Resistance to erythromycin increased from 5% in 1990, stabilizing at 11% from 1994 onwards.[12]

In order to investigate the prevalence of DRSP in Asia, the Asian Network for Surveillance of Resistant Pathogens (ANSORP) performed a study in which 996 isolates of *Str. pneumoniae* were collected from 14 participating hospitals in 12 cities, in 11 Asian countries, during September 1996 to June 1997.[13] Analysis of prevalence data for penicillin resistance revealed a rate of 80% for Korea, (the highest in the world) [13,14], followed by Japan (65%) and Vietnam (61%).[13]

Materials and Methods

All consecutive alpha-haemolytic strains isolated routinely at the Bacteriology Laboratory, St. Luke's Hospital, during the period, September 2000 through April 2002, were obtained. Samples were obtained both from in- and outpatients.

Cultures were collected in batches and tested randomly. All samples were coded, in order to protect patient identity. However, each coded sample could be traced back to the original patient.

Isolates were identified as *Str. pneumoniae* by colonial appearance and standard laboratory tests. Minimum inhibitory concentrations (MICs) were performed by Etest (AB Biodisk, Sweden) for penicillin G, erythromycin, clindamycin, ceftriaxone and vancomycin, in accordance with the manufacturer's instructions.[15] *Str. pneumoniae* ATCC 49619 was used as a control. Oxacillin susceptibility was performed using 1µg disc on Mueller Hinton Sheep Blood Agar (MH-SBA) medium (Oxoid, UK). An oxacillin disc diameter of ≤ 19mm was interpreted to be oxacillin resistant, whilst with ≥ 20mm, it was taken to be sensitive. Results were read by two independent observers.

Patients' files were traced from the Medical Records Department, after obtaining Ethics Committee approval. Relevant data such as the diagnosis at the time the sample was retrieved, concurrent medical conditions, the type of specimen, age, gender and the antibiotic treatment were noted. Such parameters were taken in order to investigate any possible correlation with DRSP and possibly help identify key factors for resistance.

Table 2: *Analysis of Isolates*

Antibiotic	Sensitive	Intermediate	Resistant
Penicillin G			
MIC Range (µg/ml)	≤0.06	0.12-1.0	≥2.0
Isolates	73% (54/74)	27% (20/74)	0
Erythromycin			
MIC Range (µg/ml)	≤0.5	1.0	≥2.0
Isolates	68.9% (51/74)	4.1% (3/74)	27 % (20/74)
Clindamycin			
MIC Range (µg/ml)	≤0.25	0.5	≥1.0
Isolates	81.1% (60/74)	0	18.9% (14/74)
Ceftriaxone			
MIC Range (µg/ml)	≤0.5	1	≥2.0
Isolates	100% (74/74)	0	0
Vancomycin			
MIC Range (µg/ml)	≤1.0	-	-
Isolates	100% (74/74)	0	0

Antibiotic Consumptions

Antibiotic consumptions for a selected group of antibiotics, namely those having activity against *Str. pneumoniae* as well as DRSP infections, were investigated. Data for the years 1997-2000 was determined. It must be stated that the values obtained were restricted only to the National Health Services. Hence, only the antibiotics, which were consumed within the Maltese Health Division, including hospitals and pharmacies, were utilized and did not include antibiotics consumed by the private sector, (i.e. community pharmacies, which number >200, private hospitals and clinics). This constitutes a limitation for the data collection.

Antibiotic consumptions were calculated in defined daily doses/1000 inhabitants/day (DID). The Maltese population statistics over the period 1997-2000 were obtained from the Demographic Review, 2000.[16] DID values for 1997 were calculated pro-rata.

Results

As can be viewed in Tables 1-2, from a total of 74 pneumococcal strains, 37 isolates demonstrated resistance to one or more of the antibiotics tested. Thirty out of sixty-two (30/62) samples were obtained from sputum, 16/62 from blood, 9/62 from throat-swabs, 5/62 from bronchoalveolar lavage (BAL) whilst 2/62 were cerebrospinal fluid (CSF) samples. However, patient data for 12 cases could not be retrieved from relevant files.

DRSP Rates

Pneumococcal resistance to penicillin was of the intermediate type (0.12-1.0µg/ml) and this amounted to 27% . Erythromycin-resistant isolates accounted for 31.1% of all pneumococcal isolates. Only 13% of these were intermediately resistant, the rest i.e. 87% showed high-level resistance, inferring ≥ 2.0µg/ml, when incubated in CO_2.[15]

The total number of clindamycin-resistant isolates equalled 18.9% and all of these were ≥ 1.0µg/ml, i.e. exhibiting high-level resistance. As was expected, clindamycin-resistant pneumococcal isolates, were also erythromycin-resistant. Hence, it can be hypothesised that the MLS_B phenotype prevails, with 61% (14/23) of erythromycin isolates. However, molecular studies should be performed, proving whether the *ermAM* gene is present in such cultures.

Patient Data vs DRSP Rates

Diagnosis at the time of sampling was obtained from patient files and classified as follows: Respiratory Disease (RD), Other Diagnosis and Unspecified. In addition, medical histories were also examined and these were limited to Cardiovascular Disease (CVD) and Diabetes Mellitus (DM). Respiratory disease accounted for 71% of DRSP cases, other diagnoses 29%, whilst 13 cases were unspecified.

Statistical analysis of the data obtained was performed by the Pearson Chi-square Test. The relationship between erythromycin resistance and RD was found to be statistically significant (p= 0.0342, n=50). In contrast, there was no statistical significance for oxacillin, penicillin and clindamycin vs diagnosis.

Interestingly, statistical analysis using the same method revealed that the relationship between CVD vs oxacillin demonstrated statistical significance (p=0.0241, n=55). In addition, the relationship between CVD vs penicillin resistance was also statistically significant (p=0.0241, n=55).

Further analysis proved that the relationship between DM vs oxacillin resistance was statistically significant (p=0.0241, n=55). This was consistent with the finding that with DM vs penicillin resistance revealed statistical significance (p=0.0137, n=55).

As *Str. pneumoniae* infections are particularly prevalent in young children (especially those ≤6 years) and in people aged ≥60 years, it was attempted to investigate whether such an association could be obtained from our study (Figure 1). However, no statistical significance was found.

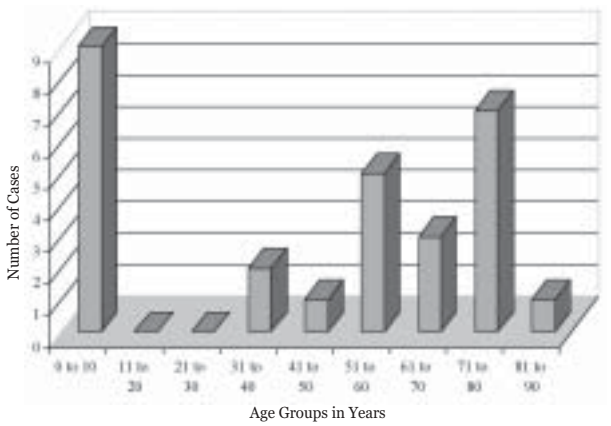
Discussion

The oxacillin disk is a screening method, which is used to detect penicillin resistance. This method offers a cheap, reliable method to distinguish between penicillin-resistant and susceptible isolates. However, it must be stated that the oxacillin disk does not discern between highly and intermediatelyresistant isolates.[17,18]

All pneumococcal isolates were subjected to oxacillin disk testing. On performing statistical analysis using the Pearson Chisquare Test (p=0.0000, n=74) it was found that the relationship between penicillin-resistant isolates and oxacillin-resistant isolates was highly significant. The findings here are thus, in accordance with the published literature.[17,18]

Since resistance to penicillin demonstrated by the pneumoccoal isolates is of the intermediate type, this infers that ß-lactams can still be advocated as first-line therapy. However, the occurrence of intermediately-resistant strains clearly

Figure 1: *Age Distribution in DRSP cases (n=28)**



**An additional 9 could not be included as, patient file records were unavailable*

denotes that a level of caution needs to be exercised and an accurate knowledge of the DRSP sensitivity patterns should be available.

This is particularly imperative, especially in certain life-threatening conditions, e.g. pneumococcal meningitis.

Third generation cephalosporins (e.g. ceftriaxone, cefotaxime) are still the mainstay of therapy, for both penicillin-intermediate and penicillin-resistant strains. In addition, researchers, advise combination therapy with vancomycin.[19-21]

As expected, statistical analysis by the Pearson Chisquare Test (p=0.0000, n=74) revealed that the relationship between clindamycin and erythromycin resistance was highly significant.

It must be noted that, from the 14 pneumococcal isolates, which exhibited high-level resistance to clindamycin, 13/14 exhibited an MIC ≥256µg/ml and only one case had an MIC of 1µg/ml (this was still considered as highly resistant).[17] Hence, it can be hypothesized that the *ermAM* gene may be found in these clindamycin-resistant pneumococcal isolates.

Montanari and co-workers[22], in Italy found that the *ermAM* gene was isolated more frequently that the *mefE* gene in erythromycin-resistant isolates. The values obtained were 76.5% for the *ermAM* gene vs 23.5% for the *mefE* gene. These findings were concordant with similar recent studies from European countries, Japan and South Africa, which also reflected the percentages, quoted by Montanari et al.[22]

Lynch refers to the finding that in certain parts of Italy and Spain *ermAM* accounts for more than 80% of macrolide-resistant strains. In the United States, efflux mechanisms account for 61% of macrolide resistance (due to *mefE*) whilst ribosomal alterations (*ermAM*) account for 32%.[8]

One of the patient criteria, which were obtained from relevant records, was antibiotic treatment. People who were given antibiotics prior to treatment, i.e., those having a history of antibiotic usage, as well as those who were given antibiotics for the current infection, i.e., at the time of sampling, were included under one heading "Antibiotic Treatment".

Statistical analysis by the Pearson Chisquare Test (p=0.0366, n=44) revealed that the relationship between antibiotic vs erythromycin resistance proved to be statistically significant. In contrast, no statistical significance was obtained for Antibiotic Treatment vs penicillin resistance.

Thorvilson and associates[23] at the Mayo Clinic, USA, compared agar dilution, broth dilution, disk diffusion and the Etest for susceptibility testing of penicillin-susceptible and penicillin-resistant *Str. pneumoniae*.

Susceptibility testing was performed on 41 clinical isolates, as well as the ATCC strain 49619 of *Str. pneumoniae.* Results showed that, overall, 71% of strains proved to be penicillin-intermediately or highly so. When comparing the methods, no serious errors (i.e., resistant strains falsely interpreted as susceptible) were observed with any of the media or any method used. Major interpretative errors (i.e., susceptible strains interpreted as resistant) were noted only with the disk-diffusion method.[23]

Hence, this study is in favour of the Etest as a reliable method for *in vitro* susceptibility testing. The study also mentioned that this test proved to be as efficient as the agar dilution and broth dilution methods.

MIC interpretation for vancomycin on Brain Heart Infusion (BHI) medium in our study, proved in most cases to be quite tedious, as growth was not always very evident on this medium, possibly due to its transparent nature and hence colonies could not be differentiated very well. AB Biodisk (Sweden) was contacted in order to ascertain whether the right medium was being used and the reply indicated that this was in fact so.

In addition, some values for vancomycin MICs, which were obtained with the Etest, were quite high i.e., an MIC of 0.75µg/ml and this is indeed very close to the limit for vancomycin susceptibility, as described by NCCLS, January 2002 of ≤1 µg/ml.

Hashemi and associates in 1996, USA[24], tested 37 clinical pneumococcal isolates, which included 13 penicillin-resistant (MIC: ≥0.125 µg/ml) and 24 penicillin-susceptible (MIC: ≤0.06 µg/ml) isolates for vancomycin susceptibility, by both the Etest and the standard microbroth dilution.

Consequently, it was demonstrated that the Etest resulted in higher MICs than those obtained with microbroth dilution. Hashemi advised that MICs obtained with the Etest, which approached 1µg/ml, should be further investigated by performing microbroth dilution.[24]

During MIC determination using the Etest, the medium used in Hashemi's study was not BHI but MH-SBA. Also, the MICs for the ATCC 49619 control strains, were outside the acceptable range on 3/5 times in Hashemi's study, but was always within the acceptable range (0.125-0.5 µg/ml) [15] for our study.

With regard to antibiotic consumptions (Table 3), ciprofloxacin, with a DID of 0.148, was the fifth most prescribed antibiotic. Even though ciprofloxacin should not be used as a first line agent for the treatment of pneumococcal infections, as evidenced by a number of studies [25-27], it was observed, whilst examining patient records, that ciprofloxacin had been prescribed to patients suffering from proven pneumococcal infections.

Cizman and co-workers[28] sought to investigate whether there was a correlation between the macrolide resistance rates for *Str. pneumoniae* and other bacteria, with the macrolide consumptions in DID over the period 1994-1999, in Slovenia. This is a small country, with an indigenous population of ca. 2 million people.

Cizman's study revealed that from 1994-1999 macrolide consumption increased from 1.89 to 3.84 DID. Interestingly, this was paralleled by an increase in resistance of *Str. pneumoniae* resistance in upper respiratory tract isolates obtained over this period. Rates increased from 0 to 9% but there was no significant increase in macrolide resistance in invasive *Str. pneumoniae* isolates during this same period.

The authors referred to the fact that in countries exhibiting

Table 3: *Average Antibiotic Consumptions (DID) over the years: 1997-2000*

Antibiotic	Average Consumption (DID)
Co-amoxicav	2.274
Amoxicillin	1.013
Erythromycin	0.344
Cephalexin	0.191
Ciprofloxacin	0.148
Roxithromycin	0.119
Cefuroxime	0.101
Co-trimoxazole	0.082
Doxycycline	0.076
Tetracycline	0.058
Phenoxymethyl penicillin	0.052
Clindamycin	0.052
Clarithromycin	0.049
Rifampin	0.040
Ceftriaxone	0.031
Benzyl penicillin	0.012
Meropenem	0.010
Cefotaxime	0.010
Teicoplanin	0.007
Imipenem & cilastatin	0.005
Vancomycin	0.004
Azithromycin	0.002
Cephalothin	0.001
Benzathine penicillin	0.001

low macrolide consumptions (0.8-2.0 DID), as in Scandinavian countries, the prevalence of resistance in *Str. pneumoniae* was low (<10%), whereas in countries where macrolide consumptions were high (>3.6 DID), typically France and Spain, resistance rates were correspondingly high.

Hence, Cizman and associates concluded that a two-fold increase in the macrolide consumption during the study period 1994-1999, in Slovenia, was associated with a nearly linear increase in macrolide resistance in upper respiratory *Str. pneumoniae* isolates.[28]

In conclusion, local resistance rates presented here are definitely an eye-opener. Indeed, following our study, a DRSP surveillance unit was established in order to monitor such patterns closely. Ideally, studies like ours should be conducted over a period of 5-10 years and the data collected correlated with significant patient factors. Serotyping of all pneumococcal isolates should be encouraged, especially, in view of the availability of vaccines and the possibility of acquiring/ developing improved preparations. There should also be an investment in molecular genetic techniques, in order to clearly identify specific genes associated with resistance. Finally, stricter antibiotic policies should be enforced; thereby, reducing the spread of resistance.

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