Session V
Treatment of Gonorrhœa

Chairman Dr A Percival

Activity *in vitro* of Cefuroxime and Six Other Antimicrobial Agents against *Neisseria gonorrhœæ* isolated in Belgium and Rwanda

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Although a gradual increase in chromosomal resistance to penicillin has been observed for *Neisseria gonorrhœæ* in the last decades, penicillin in large doses remained a highly efficient and economical drug in the treatment of gonococcal infections (Kaufman *et al.* 1976, Jaffe *et al.* 1976). Alternative therapeutic regimens were used for treatment failures and in penicillin hypersensitive patients. But since the emergence in 1976 of β-lactamase producing *N. gonorrhœæ* in many parts of the world (Ashford *et al.* 1976, Phillips *et al.* 1976) there is an urgent need for alternative penicillinase-stable antibiotics in the treatment of gonorrhœa.

Since 1973 our laboratory has been involved in a surveillance programme on the antibiotic susceptibility of *N. gonorrhœæ* in Belgium (Meheus *et al.* 1976). Because of its stability against β-lactamase and its reported activity *in vitro* against gonococci (O'Callaghan *et al.* 1976), cefuroxime was included in the most recent survey of Belgian strains. For comparison, and because recent data on antibiotic susceptibility of *N. gonorrhœæ* from Africa are rare, strains from Rwanda (Central Africa) were simultaneously tested.

MATERIALS AND METHODS

Strains

One hundred and eighty-nine unselected strains of *N. gonorrhœæ* from Antwerp (136 strains) and from Leuven and Gent (53 strains) were collected between March 1975 and March 1977, and were preserved by lyophilization after testing for the presence of β-lactamase. The 53 Rwandese strains were isolated in 1976 in Kigali and Butare. Identification was based on colonical morphology, Gram stain and growth enhancement in a 10% CO_2 enriched atmosphere. Each strain was tested for oxidase and β-galactosidase activity and for fermentation of glucose, maltose, sucrose and lactose.

N. gonorrhœæ WHO reference strains III, V and VII (A Reyn, Statens Seruminstitut), *Staphylococcus aureus* ATCC 25923 and *N. gonorrhœæ* 1916E (Glaxo) were included in each experiment.

Antibiotics

National standards of benzylpenicillin, ampicillin, erythromycin and tetracycline hydrochloride were provided by the Laboratory of Pharmaceutical Standards, Ministry of Health, Brussels. Sulfamethoxazole and trimethoprim were supplied by Roche Products Ltd, spectinomycin by Upjohn Ltd, and cefuroxime by Glaxo Group Ltd. The combination sulphamethoxazole/trimethoprim (SMZ/TMP) was tested in the ratio 19:1.

Table 1

Distribution of minimal inhibitory concentrations (MIC) for 189 strains of *Neisseria gonorrhoœæ* isolated in Belgium 1976–1977

Antibiotic	*Median value of MIC (μg/ml)*	*MIC (μg/ml)*													
		<0.0078	*0.0156*	*0.0312*	*0.0625*	*0.125*	*0.25*	*0.5*	*1*	*2*	*4*	*8*	*16*	*32*	*64*
Penicillin	0.015	101	11	10	26	37	3								1
Ampicillin	0.058	3	38	63	16	41	24	3							1
Erythromycin	0.081		2	62	104	11	7	1		2					
Tetracycline	0.72						63	71	54	1					
Spectinomycin	13.66										7	123	59		
Cefuroxime	0.018	87	59	28	7	5	3								
Sulfamethoxazole[a]/ trimethoprim 19/1	9.33								2	37	46	60	35	9	

[a] = MIC is expressed as μg/ml of the mixture

Minimal Inhibitory Concentration (MIC)

With a multipoint replicator, suspensions from overnight cultures in 5 ml of blood saponin broth (Tryptic soy broth [Difco 0370]; 1% saponin [Merck 7695] plus 5% horse blood) were inoculated on to plates containing the antibiotics (DST agar [Oxoid CM 261]; 1% saponin [Merck 7695] plus 5% horse blood). The inoculum was 10^4 colony forming units (CFU). Control plates without antibiotics were inoculated as the first and last plates in each series. After incubation for 24 h the minimum inhibitory concentration (MIC) was determined as the lowest concentration of each antibiotic in which bacterial growth was completely inhibited, or the growth of a maximum of 5 colonies occurred.

Statistical Methods

Rank correlation coefficients were determined with Spearman's rank correlation test.

Results

The distribution of MICs for the Belgian and the Rwandese strains is shown in Tables 1 and 3 respectively, together with the median values for each antibiotic. A highly penicillin-resistant *N. gonorrhœæ* was isolated in Antwerp in March 1977 and found to be directly imported from the Ivory Coast (Piot 1977). Table 2 gives the MICs for this strain, whose β-lactamase was characterized as a TEM-1 type enzyme.

On a weight basis, cefuroxime was as active as penicillin against the strains from Belgium and more active against the Rwandese strains. The median value for cefuroxime of the latter is less than one-third of the value for penicillin. Fig 1 shows the unimodal distribution of the MICs for cefuroxime in both populations, with the mode at 0.0078 μg/ml for the Belgian gonococci and at 0.0156 μg/ml for the Rwandese strains. At the 0.0312 μg/ml level, 91% of the former and 85% of the latter were inhibited. As for penicillin, the African gonococci were less susceptible to cefuroxime than the isolates from Belgium. All strains were inhibited by 0.25 μg/ml of cefuroxime.

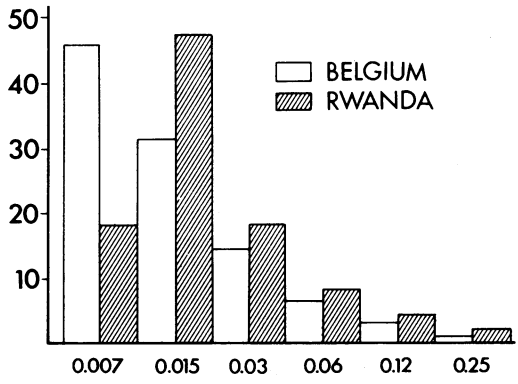

Fig 1 *Distribution of MIC values of cefuroxime against* Neisseria gonorrhœæ *from Belgium and Rwanda. % of strains (ordinate) is plotted against MIC*

The well-known bimodal distribution is found for the MICs for penicillin and ampicillin both in Belgium and Rwanda, with the first and the second modes at the same MIC. However, the Rwandese strains are less susceptible to penicillin, as illustrated by their higher median value. About 57% of the strains from Rwanda are less susceptible to penicillin (MIC≥0.06 μg/ml), compared to 35% of the strains from Belgium, but except for the β-lactamase producing strain from Antwerp, the highest MIC value was not more than 0.25 μg/ml. All strains from both countries were sensitive to spectinomycin. One strain from Rwanda had an MIC of 8 μg/ml of tetracycline and the MIC of SMZ/TMP for another strain was 64 μg/ml.

Table 2

Minimal inhibitory concentrations (MIC) of a β-lactamase producing *Neisseria gonorrhœæ* isolated in Antwerp (μg/ml)

Benzylpenicillin	64
Ampicillin	64
Cefuroxime	0.0312
Tetracycline hydrochloride	1
Spectinomycin	8
Sulphamethoxazole/trimethoprim (19/1)	3.8/0.2

Table 3

Distribution of minimal inhibitory concentrations (MIC) for 53 strains of *Neisseria gonorrhœæ* isolated in Rwanda 1976

Antibiotic	*Median value of MIC (μg/ml)*	*MIC (μg/ml)*													
		≤0.0078	*0.0156*	*0.0312*	*0.0625*	*0.125*	*0.25*	*0.5*	*1*	*2*	*4*	*8*	*16*	*32*	*64*
Penicillin	0.090	14	3	6	9	16	5								
Ampicillin	0.144		1	15	8	20	9								
Erythromycin	0.090			19	18	8	7			1					
Tetracycline	0.88						8	25	18	1		1			
Spectinomycin	14.50										1	32	20		
Cefuroxime	0.026	10	25	10	5	2	1								
Sulphamethoxazole[a]/ trimethoprim 19/1	10.80									6	14	20	11	1	1

[a] = MIC is expressed as μg/ml of the mixture

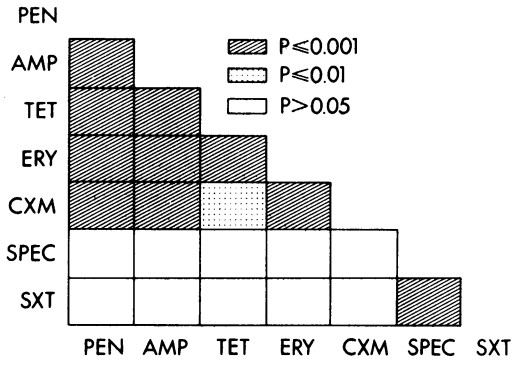

Fig 2 *Rank correlation coefficients between MICs for* Neisseria gonorrhœæ *from Rwanda (53 strains). PEN=penicillin; AMP=ampicillin; TET=tetracyline; ERY=erythromycin; CXM=cefuroxime; SPEC=spectinomycin; SXT=sulphamethoxazole/trimethoprim*

Fig 2 shows a simplified diagram of the 21 rank correlation coefficients between the MICs for the Rwandese strains. A significant positive correlation ($P \leq 0.01$) was found between the sensitivities to penicillin, ampicillin, erythromycin, tetracycline and cefuroxime. No correlation was found between the sensitivities to spectinomycin and to SMZ/TMP, except for the pair spectinomycin – SMZ/TMP.

Discussion

Despite increasing efforts for the detection of penicillin-resistant gonococci, no other penicillinase-producing *N. gonorrhœæ* has been reported in Belgium since the isolation of the single strain in Antwerp.

The incidence of penicillinase-producing strains of *N. gonorrhœæ* in Belgium seems very low, but there is a continual danger of import of these strains from high-prevalence areas, such as the Far East and West Africa. Although the sample from Rwanda is rather limited, it suggests that penicillinase-producing gonococci are rare in Belgium.

Surprisingly, the overall susceptibility pattern for penicillin in Rwanda compared favourably with other studies from Africa (Arya & Lawson 1977), where there is a high prevalence of chromosomally-mediated penicillin resistance. The discordance may be due to differences in methods, although our results for the WHO reference strains are not in agreement with this explanation.

As expected, the Rwandese strains show a decreased sensitivity to penicillin, compared with the Belgian strains. Particular epidemiologic parameters such as local treatment schedules, the widespread practice of self-medication, and individual antibiotic usage may influence susceptibility patterns of gonococci.

Cefuroxime, *in vitro* at least, was as active as penicillin against *N. gonorrhœæ*, as found earlier by Phillips *et al.* (1976) in a comparative study on the action of different cephalosporins on gonococci. However, a bimodal distribution for cefuroxime) as they reported) was not found in our material.

Results from the rank correlation coefficient determinations show a positive correlation between the susceptibilities to cefuroxime and to penicillin in a population of non-penicillinase producing gonococci, as illustrated by the higher MICs of cefuroxime for the strains from Rwanda, where the penicillin sensitivity is lower than in Belgium. However, in the former population, cefuroxime was more active than penicillin, as shown by the distribution of MICs and their median values. By regression coefficient analysis Phillips (1976) also found that cefuroxime was more active than benzylpenicillin against penicillin resistant strains, but that MICs for cefuroxime were higher with decreased penicillin susceptibility.

The stability of cefuroxime to the β-lactamase of *N. gonorrhœæ* has already been reported (Phillips 1976, Percival *et al.* 1976) and is illustrated by the only penicillinase producing strain included in this study. Preliminary results from Liverpool (Percival *et al.* 1976) suggest that cefuroxime deserves further clinical investigation in gonococcal infections. Since there is a clear possibility of eventual spectinomycin resistant gonococci (McCormack & Finland 1976), alternative drugs for the treatment of penicillinase producing *N. gonorrhœæ* infections are needed. These alternative agents may include enzyme stable cephalosporins, if their *in vitro* efficacy is confirmed in clinical trials.

Summary

One hundred and eighty-nine Belgian strains and 53 Rwandese strains of *N. gonorrhœæ* were tested for their sensitivity to cefuroxime, benzyl penicillin, ampicillin, erythromycin, tetracycline hydrochloride, spectinomycin and a combination of sulphamethoxazole and trimethoprim, in a 19:1 ratio. Distribution and median values of minimum inhibitory concentrations (MIC) are given and discussed. Low level resistance for penicillin (MIC>0.0625 μg/ml) was observed in 35% of Belgian and 57% of the Rwandese strains. Cefuroxime was very active against all strains, the Rwandese isolates being less sensitive than the Belgian isolates. Of the Belgian strains, 46% were inhibited by 0.0078 μg/ml of cefuroxime, 94% by 0.0312 μg/ml and all strains by 0.25 μg/ml. The median value for the Belgian isolates was 0.0179 μg/ml (compared to 0.015 μg/ml for

penicillin) and 0.026 μg/ml for the Rwandese isolates (0.090 μg/ml for penicillin). A penicillinase producing *N. gonorrhœæ*, isolated in Belgium had a MIC for penicillin of 64 μg/ml and for cefuroxime of 0.03125 μg/ml.

For the non-penicillinase producing strains, a significant positive correlation ($P<0.01$, rank correlation coefficient) was found between the sensitivities to cefuroxime, penicillin, ampicillin, erythromycin and tetracycline, but not between cefuroxime and spectinomycin and the combination sulphamethoxazole/trimethoprim.

Acknowledgment

We are indebted to Dr Simpson, Glaxo Ltd for characterization of the β-lactamase and to Mr Bosmans, Centre Hospitalier, Kigali, for the Rwandese strains of *N. gonorrhœæ*.

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