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Antimicrobial Susceptibility (S) and Epidemiology of a Worldwide Collection of *Chryseobacterium* spp.: Report from the SENTRY Antimicrobial Surveillance Program (1997-2001)

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SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT

Background: Members of *Flavobacterium* spp. have been classified under the new genus *Chryseobacterium* (CHRY). Limited data are available on these pathogens leading to an evaluation of patient demographics and S patterns for CHRY collected in the SENTRY Program (5 years).

Methods: Only clinically significant isolates were selected. Identification was confirmed by the monitor laboratory and S testing was performed by NCCLS methods. Strains were screened for metallo-ß-lactamase (MßL) by Etest and MßL activity was confirmed by meropenem (MEM) hydrolysis assay and inhibition with EDTA.

Results: 50 isolates from Europe (5), North America (15), Latin America (14) and Asia-Pacific (16) were collected and included 24 (48%) *C. meningosepticum* (CMEN), 20 (40%) *C. indologenes* (CHIN), 2 (4%) *C. gleum* and 4 (8%) unidentified CHRY spp. CHRY isolates represented only 0.27% of non-fermentors and 0.03% of the total SENTRY collection. The highest CHRY prevalence was detected in the elderly (0.045%) and lowest among children (0.016%). CMEN was most frequently isolated from bloodstream (BSI; 54%) > respiratory tract (RTI; 42%) > skin and soft tissue (4%). CHIN was more frequent in RTI (70%) > BSI (30%). 43% of the patients were hospitalized in general medicine units. The most active drugs were newer fluoroquinolones (FQs; garenoxacin, gatifloxacin and levofloxacin; MIC_{50} at 1 µg/ml and 98% S) followed by rifampin (MIC_{90}, 2 µg/ml). Trim/sulfa (88% S), ciprofloxacin (80%) and piperacillin/tazobactam (80%) were also active. Often recommended vancomycin showed poor potency (MIC_{50}, 16 µg/ml) versus CHRY. MßL activity (average MEM hydrolysis: 529 µmol/min/mg) was demonstrated in all isolates tested.

Conclusions: CHRY mainly caused BSI and RTI in the elderly. MßLs were intrinsic to CHRY and the best therapeutic option appeared to be the newer or investigational FQs.

INTRODUCTION

Ubiquitous in nature, *Chryseobacterium* species are found primarily in soil and water. Environmental studies have revealed that these organisms can survive in chlorine treated municipal water supplies often colonizing sink basins and taps creating potential reservoirs for infections inside hospital environs. Frequent colonization of patients via fluid contaminated medical devises (respirators, intubation tubes, mist tents, humidifiers, newborn incubators, ice chests, syringes etc.) has been documented. Contaminated surgically implanted devises such as intravascular catheters and prosthetic valves have also been reported.

Chryseobacteria have been described as etiological agents of meningitis, bacteremia, pneumonia, endocarditis, skin and soft tissue, ocular and others infections. Primarily opportunistic pathogens, they mainly infect newborns and immunocompromised hosts from all age groups.

Antimicrobial susceptibility data on *Chryseobacterium* spp. remains very limited since this pathogen has been rarely isolated from clinical specimens. In addition, results of susceptibility testing vary when different methods are used. Results from disk diffusion methods may not be reliable and broth reference-quality microdilution tests should be performed when possible.

The SENTRY Antimicrobial Surveillance Program is a world-wide study monitoring the susceptibility and resistance patterns of bacterial and fungal pathogens. This investigation was conducted using results from over 119 sentinel hospitals and laboratories in North America, Latin America, Europe and the Asia-Pacific region from the initial five years of the program (1997-2001). During this time period over 155,811 clinical isolates were collected from several sites of infections, including bloodstream, lower respiratory tract, skin and soft tissue and urinary tract. In this collection, 50 *Chryseobacterium* isolates were identified and selected for detailed characterization and additional antimicrobial susceptibility testing.

MATERIALS & METHODS

Study design. All *Chryseobacterium* spp. isolates collected from the SENTRY Program during 1997-2001 were evaluated. Individual strains came from hospitalized patients in four international regions: Asia-Pacific region (16 strains from seven centers), Europe (5 strains from five centers), Latin America (14 strains from six centers) and North America (15 strains from 15 centers). A summary of demographic data, including age, sex, ward, and hospitalization in the intensive care unit, was also obtained.

Antimicrobial susceptibility. Each strain was tested against 47 antimicrobial agents by broth microdilution method (dry-form panels [TREK Diagnostic Systems, Inc., Cleveland, OH]) as specified by the NCCLS. Interpretation of MIC results was in accordance with NCCLS criteria. Selected Gram-positive-active drugs tested against *Chryseobacterium* species isolates were interpreted by breakpoints approved for *Staphylococcus* or *Enterococcus* species.

Molecular Typing. Multiple isolates of the same species isolated by the same medical center were typed by pulsed-field gel electrophoresis after digestion of genomic DNA with *Spe* I.

Metallo-ß-Lactamase Activity. Meropenem hydrolysis was evaluated in 21 randomly selected strains (11 *C. indologenes* and 10 *C. meningosepticum*) by UV spectrophotometric assays (Pharmacia LKB Ultrospec II) in 1-cm light path cuvettes with readings recorded at 10-s intervals for five min at a wavelength of optimal absorbence (299 nm). The antimicrobial solution was prepared in 10 µM phosphate buffer, pH 7.0. For the substrate profile, meropenem was assayed at a concentration of 100 µM. The inducibility was assessed with cefoxitin at 0.25 µg/ml, challenged for two hours.

COMMENTS

- The 50 isolates were collected from 33 medical centers in 16 countries.
- *Chryseobacterium* spp. represented only 0.27% (50/18,569) of the processed non-fermentative Gram-negative bacilli, and 0.03% (50/155,811) of all bacterial isolates collected by the SENTRY Program during the five-year period evaluated (1997-2001).
- The most frequently isolated species was *C. meningosepticum* (24 isolates, 48%), followed by *C. indologenes* (20 isolates, 40%) and *C. gleum* (2 isolates, 4%) and *Chryseobacterium* spp. (8%). All isolates were from hospitalized patients and the vast majority was recovered from the lower respiratory tract (26 isolates, 52%) and blood cultures (23 isolates, 46%).
- The frequency of *Chryseobacterium* among respiratory tract specimens (0.10%, 26 out of 25,657 specimens evaluated) was three-fold higher than the rate among positive blood cultures (0.03%, 23 out of 74,236).
- Among isolates from bloodstream infections, 52.2% were *C. meningosepticum* and 30.4% were *C. indologenes*. Conversely, half of the isolates from the respiratory tract were *C. indologenes* and 42.3% were *C. meningosepticum*.
- The highest frequency of *Chryseobacterium* spp. infection occurred among the elderly (≥ 65 years old; 0.045%) and the lowest among children ≤ 5 years of age (0.016%).
- The quinolones showed the highest potency and spectrum of activity against this collection of *Chryseobacterium* spp.
- Garenoxacin was the most active compound (MIC_{50}, 0.12 µg/ml; MIC_{90}, 1 µg/ml), and this new desfluoroquinolone inhibited 98.0% of isolates at the proposed susceptible breakpoint (≤ 2 µg/ml) [Howard et al., 2002]. Gatifloxacin (MIC_{50}, 0.25 µg/ml) and levofloxacin (MIC_{50}, 0.5 µg/ml) also inhibited 98.0% of the isolates at susceptible breakpoints, and the susceptibility rate to ciprofloxacin (MIC_{90}, 0.5 µg/ml) was significantly lower (80.0%).
- Trimethoprim/sulfamethoxazole (87.8% susceptibility [S]), rifampin (MIC_{50}, 0.5 µg/ml; 85.7% S) piperacillin/tazobactam (MIC_{50}, 4 µg/ml; 80.0% S), piperacillin (MIC_{50}, 8 µg/ml; 74.0% S) and cefepime (MIC_{50}, 4 µg/ml; 62.0% S) showed reasonable activity. All other non-quinolone compounds tested showed poor activity against these pathogens.
- Isolates from the Asia-Pacific region showed higher resistance rates to the ß-lactams. Susceptibility rate to piperacillin/tazobactam was only 50% in the Asia-Pacific region (16 isolates) compared to 85.7 - 100% in the other regions evaluated. Susceptibility rates did not vary greatly among regions for other classes of antimicrobial agents.
- Metallo-ß-lactamase activity (meropenem hydrolysis) was demonstrated for all isolates evaluated with activity ranging from 381 to 788 (average 529) µmol/min/mg of protein (Table 4).
- Three *C. indologenes* isolates from a Brazilian center had identical PFGE pattern (Figure 1). These isolates where collected within a 10-day period from the lower respiratory tract of elderly patients (ages 76 – 90 years) with nosocomial pneumonia hospitalized in the intensive care unit (ICU).
- Two *C. meningosepticum* isolates from the same Brazilian medical center shared a unique PFGE pattern (Figure 1). These strains were also isolated in the ICU from elderly patients (ages, 66 and 69 years) from bloodstream infection and the lower respiratory tract (hospital-acquired pneumonia).

Table 1. In vitro activity of selected antimicrobials against 50 *Chryseobacterium* spp.[a] strains from the SENTRY Program (1997-2001).

	MIC (µg/ml)		% category:	
Antimicrobial agent[b]	50	90	Susceptible	Resistant
Garenoxacin[c,d]	0.12	1	98.0	0.0
Gatifloxacin	0.25	1	98.0	0.0
Levofloxacin	0.5	1	98.0	2.0
Ciprofloxacin	0.5	>2	80.0	12.0
Trimethoprim/Sulfamethoxazole[d]	≤0.5	>2	87.8	12.2
Piperacillin/Tazobactam	4	64	80.0	4.0
Piperacillin	8	128	74.0	20.0
Ticarcillin/Clavulanate	>128	>128	4.0	74.0
Cefepime	4	>16	62.0	24.0
Ceftazidime	>16	>16	46.0	52.0
Ceftriaxone	32	>32	22.0	26.0
Imipenem	>8	>8	12.0	88.0
Meropenem	>8	>8	6.0	86.0
Amikacin	32	>32	14.0	42.0
Gentamicin	>8	>8	8.0	88.0
Tobramycin	>16	>16	0.0	100.0
Chloramphenicol[d,e]	>16	>16	4.1	81.6
Clindamycin[d,e]	4	8	10.2	53.1
Linezolid[d,e]	>8	>8	8.1	-
Teicoplanin[d,e]	16	>16	12.2	46.9
Vancomycin[d,e]	16	16	2.0	10.2
Rifampin[d,e]	0.5	2	85.7	0.0

a. Includes: *C. gleum* (two strains), *C. indologenes* (20 strains), *C. meningosepticum* (24 strains) and *Chryseobacterium* spp. (four strains).
b. NCCLS MIC breakpoints for non-Enterobacteriaceae were categorically applied to *Chryseobacterium* spp. unless otherwise noted.
c. A susceptible breakpoint of ≤ 2 µg/ml was applied.
d. One strain of *C. gleum* was unavailable for testing against this compound.
e. NCCLS breakpoints for *Staphylococcus* spp. were applied.

Table 2. MIC result distribution for the most active antimicrobials against all *Chryseobacterium* spp. from the SENTRY Program (1997 - 2001).

	No. of isolates (cumulative %) inhibited at MIC (µg/ml) of:										
Antimicrobial agent[a]	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64
Garenoxacin[b]	10(20)	5(31)	8(47)	11(69)	8(86)	5(96)	1(98)	1(100)	-[c]	-	-	-
Gatifloxacin	3(6)	3(12)	12(36)	10(56)	12(80)	6(92)	3(98)	1(100)	-	-	-	-
Levofloxacin	-	-	-	-	37(74)	8(90)	4(98)	-	-	-	-	-
Ciprofloxacin	-	1(2)	2(6)	7(20)	20(60)	10(80)	4(88)	-	-	-	-	-
Trimethoprim/Sulfamethoxazole	-	-	-	-	25(51)	6(63)	12(88)	-	-	-	-	-
Piperacillin/Tazobactam	-	-	-	-	5(10)	1(12)	10(32)	11(54)	7(68)	6(80)	0(80)	8(96)
Cefepime	-	-	3(6)	3(12)	9(30)	7(44)	2(48)	1(50)	6(62)	7(76)	-	-
Ceftazidime	-	-	-	-	-	-	13(26)	8(4)	2(46)	1(48)	-	-

a. NCCLS MIC breakpoints for non-Enterobacteriaceae were applied to unless otherwise noted. Underlined values indicate susceptible breakpoint applied and percentage of susceptible strains.
b. A susceptible breakpoint of ≤ 2 µg/ml was applied. One strain of *C. gleum* was unavailable for testing against this compound.
c. - Not tested.

Table 3. Spectrum of selected antimicrobial agents against the most frequently isolated species.

	C. meningosepticum (24)		*C. indologenes* (20)	
Antimicrobial agent[a]	% susceptible	% resistant	% susceptible	% resistant
Garenoxacin[b]	100.0	0.0	95.0	0.0
Gatifloxacin	100.0	0.0	95.0	0.0
Levofloxacin	95.8	4.2	100.0	0.0
Ciprofloxacin	70.9	16.7	85.0	10.0
Trimethoprim/Sulfamethoxazole	79.2	20.8	95.0	5.0
Piperacillin/Tazobactam	71.0	4.2	90.0	5.0
Piperacillin	62.4	29.2	85.0	10.0
Cefepime	37.6	33.0	85.0	15.0
Ceftazidime	4.2	91.7	85.0	15.0
Imipenem	0.0	95.8	15.0	85.0
Amikacin	4.2	62.5	15.0	20.0
Rifampin[c]	87.5	0.0	85.0	0.0

a. NCCLS MIC breakpoints for non-Enterobacteriaceae were categorically applied to *Chryseobacterium* spp.
b. A susceptible breakpoint of ≤ 2 µg/ml was applied.
c. NCCLS MIC breakpoints for *Staphylococcus* spp. were applied.

Table 4. Metallo-ß-lactamase activity of selected strains measured by meropenem hydrolysis activity.

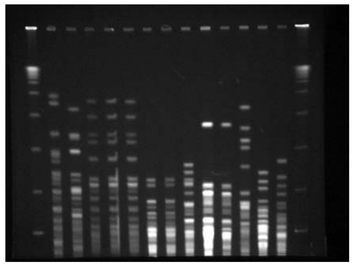
Isolate number	Species	Meropenem hydrolysis activity (µmol/min/mg of protein)
004-136	*C. indologenes*	623
019-1839	*C. indologenes*	381
021-2707	*C. indologenes*	604
035-2184	*C. indologenes*	586
041-3697	*C. indologenes*	537
041-3700	*C. indologenes*	511
041-3767	*C. indologenes*	502
044-1725	*C. indologenes*	485
046-3338	*C. indologenes*	419
049-2066	*C. indologenes*	655
085-9091	*C. indologenes*	610
013-9794	*C. meningosepticum*	604
022-1442	*C. meningosepticum*	448
027-785	*C. meningosepticum*	576
029-2252	*C. meningosepticum*	788
037-3826	*C. meningosepticum*	395
041-2981	*C. meningosepticum*	592
041-3541	*C. meningosepticum*	398
044-5371	*C. meningosepticum*	441
049-7561	*C. meningosepticum*	553
055-5152	*C. meningosepticum*	414

CONCLUSIONS

- *Chryseobacterium* generally caused BSI and RTI in the elderly.
- The finding of two small epidemic clusters involving elderly patients hospitalized in the ICU with lower respiratory tract infections raises the concern of the occurrence of outbreaks in this patient population.
- The production of metallo-ß-lactamase was intrinsic to *Chryseobacterium*.
- The newer quinolones (garenoxacin, gatifloxacin, levofloxacin) appear to be the most appropriate antimicrobial agents to treat infections caused by this pathogen.
- Extensive world-wide surveillance programs such as the SENTRY Program, are extremely important to guide empiric antimicrobial therapy and clinical context of rarely isolated pathogens.

Figure 1: Chromosomal patterns (PFGE) of *Chryseobacterium* isolates from medical centers that had multiple isolates. Colomns 1 to 5 represent *C. indologenes* and columns 6 to 16 represent *C. meningosepticum*. Columns 3 to 7 represent isolates from elderly patients hospitalized in an ICU from a Brazilian medical center (two clusters at center 41), while columns 9 and 10 represent isolates from a unique patient. λ:lambda ladder 48.5 kb.

λ 1 2 3 4 5 6 7 8 9 10 11 12 13 λ



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