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Case Report

A case of suspected infective endocarditis with *Lactococcus garvieae*: lack of *in vitro* synergy between ampicillin and gentamicin

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Introduction: *Lactococcus garvieae* is an uncommon cause of infective endocarditis (IE) and, despite the fact that synergy between beta-lactam antibiotics and aminoglycosides has not been demonstrated for lactococci, combination therapy is often used.

Case presentation: We report a case of suspected *L. garvieae* IE in an 82-year-old man, which was successfully treated with a combination of ampicillin and gentamicin. Despite careful dosing and monitoring of gentamicin levels, the patient developed a transient decrease in renal function and permanent bilateral vestibular deficiency. The combination of gentamicin and ampicillin did not demonstrate synergistic killing effects *in vitro* against the *L. garvieae* isolate from this patient. However, synergy was noted against two of the four *L. garvieae* isolates tested. Unfortunately, an Etest-based method, which would be easy to use in a routine laboratory, was unable to predict synergy in the time-kill methods.

Conclusions: The use of combination therapy in IE is based solely on *in vitro* synergy between beta-lactams and aminoglycosides. Here we demonstrate that a combination of ampicillin and gentamicin induces synergistic killing only of some *L. garvieae* isolates. Since synergy does not seem to be omnipresent, the risks for aminoglycoside toxicity must be carefully weighed against the potential theoretical benefit of combination therapy in *L. garvieae* IE.

Keywords: infective endocarditis; *Lactococcus garvieae*.

Introduction

Lactococcus garvieae is a Gram-positive, catalase-negative bacterium, growing in pairs or in short chains. Infective endocarditis (IE) caused by *Lactococcus* sp. was first described in 1955 (Wood *et al.*, 1955) and since then a small number of IE cases have been reported with *Lactococcus lactis* (Rostagno *et al.*, 2013) and *L. garvieae* (Rasmussen *et al.*, 2014; Russo *et al.*, 2012). Infections caused by *L. garvieae* have been proposed to be mainly transmitted to humans from contaminated fish (Wang *et al.*, 2007), and IE seems to be a common presentation of infection with the bacterium (Chan *et al.*, 2011). IE caused by *L. garvieae* most often affects prosthetic valves, but native valve IE has also been reported (Russo *et al.*, 2012). A combination of beta-lactams and aminoglycosides is commonly used to treat lactococcal IE (Rasmussen *et al.*, 2014; Rostagno *et al.*, 2013; Russo *et al.*, 2012), based on the presumption of a synergistic killing effect as has been described for enterococci and viridans streptococci (Farber *et al.*, 1983; Winstanley & Hastings, 1990). An aminoglycoside and beta-lactam synergistic killing effect on lactococci has to our knowledge not been demonstrated. Thus, the use of aminoglycosides in lactococcal IE lacks even a theoretical base and since aminoglycosides are toxic their use should be well justified. Here we describe a case of suspected IE with a *L. garviaeae* isolate, against which there was no synergism between the beta-lactam and gentamicin, where the patient had severe side-effects related to gentamicin treatment.

Abbreviations: CRP, C-reactive protein; IE, infective endocarditis; FIC, fractional inhibitory concentration; MBC, minimal bactericidal concentration.

Case presentation

An 82-year-old man was referred to the Helsingborg Hospital due to malaise and intermittent fever for 2 weeks. He had a medical history of thoracic surgery due to constrictive pericarditis 15 years earlier and a chronic atrial fibrillation, and he had a pacemaker owing to brady-tachy syndrome. He had declined warfarin treatment and was only on medication with aspirin (75 mg) and metoprolol (50 mg) daily. At presentation, the patient was afebrile but tachycardia (140 beats min^{-1}) was noted. There was no cardiac murmur, nor signs of septic embolization. Laboratory testing revealed a C-reactive protein (CRP) of 52 $mg\,l^{-1}$

and a slightly elevated serum creatinine of 120 μmol l^{-1}, the latter being at the patient's baseline level. Three blood cultures were taken, but antibiotics were withheld as there was no confirmed fever and the patient was deemed stable. A computerized tomography (CT)-scan was performed and it excluded the possibility of pulmonary embolism.

The following evening, a temperature of 38.0 °C was noted and two new blood cultures were collected. All five sets of blood cultures grew Gram-positive cocci in chains, and empirical treatment with benzyl-penicillin and gentamicin was instituted. The bacteria were later identified as *L. garvieae* using 16S rRNA gene PCR and sequencing. The patient denied having consumed or handled raw or poorly cooked fish. The patient was transferred to the department for infectious diseases and treatment was changed to ampicillin and gentamicin. A transthoracic echocardiogram showed a non-significant mitral regurgitation, and valves and the pacemaker cables showed no sign of vegetation. A transoesophageal echocardiogram was also performed, without objective signs of endocarditis. Although the patient only fulfilled one major (persisting bacteraemia) and two minor (fever and presence of pacemaker) Duke criteria it was decided that he was to receive IE treatment with 2 weeks of combined beta-lactam and aminoglycoside, followed by 2 weeks of a beta-lactam antibiotic as a mono-therapy. Etest results from the microbiology laboratory demonstrated that the isolate had a MIC of 0.25 mg l^{-1} for ampicillin, 0.5 mg l^{-1} for PcG and 2 mg l^{-1} for gentamicin. Ampicillin 3 g four times daily and gentamicin 2.5 mg kg^{-1} day^{-1} (national guidelines suggests 3 mg kg^{-1} day^{-1}, available at http://www.infektion.net) were continued in order to achieve a potential synergistic effect. The CRP level decreased to normal levels within the first week and the patient gradually felt better. On the seventh and eighth day a temperature of 38 °C was noted, but thereafter he was afebrile. Serum levels of gentamicin were measured every third day as trough values and peak values, never exceeding 1.0 mg l^{-1} and 12.1 mg l^{-1}, respectively. Despite this, the gentamicin dose had to be reduced twice (1.5 mg kg^{-1} day^{-1} for the last three days) owing to gradually increasing levels of creatinine (maximum 197 μmol l^{-1} just after the discontinuation of gentamicin). The patient remained hospitalized for another 2 weeks to receive ampicillin treatment and during this period creatinine levels decreased to 160 μmol l^{-1}. However, the patient started to develop unsteadiness, which became apparent 1 week after gentamicin had been discontinued. No other neurological symptoms except for a broad-based gait and a slight imbalance while performing Romberg's manoeuvre were noted. A permanent bilateral vestibular deficiency was confirmed 2 weeks after discharge in the follow-up at the otorhinolaryngeal outpatient clinic. At this follow-up, serum creatinine levels had decreased to the baseline level, but the patient had developed a chronic disability and was now requiring a cane to walk. The patient moved to another province and was lost to further follow-up.

Investigations

The isolate from the patient described, as well as three other *L. garvieae* blood isolates from a patient with diverticulitis, a patient with IE (Rasmussen *et al.*, 2014), and an infant with no obvious focus of infection were tested for possible synergistic effects between ampicillin (Sigma) and gentamicin (Schering-Plough). We used both time-kill experiments and Etest-based methods to detect possible synergy. First, the minimal bactericidal concentration (MBC) for gentamicin and ampicillin was measured using serial microdilution in MH-F medium as described (http://www.eucast.org). Bacteria were plated on blood agar after 24 h incubation at 37 °C and 5 % CO_2. MBC for ampicillin was found to be 1 mg l^{-1} for all isolates, whereas MBC for gentamicin was either 2 or 4 mg l^{-1} (Table 1). Bacterial killing was determined as described in Weinstein & Moellering (1975). The bacteria were from blood agar plates incubated overnight, and were diluted in PBS to 0.5 McFarland, and then diluted 1 : 100 in MH-F broth (as described at http://www.eucast.org). Ampicillin concentrations of 0.5 and 1 mg l^{-1}, together with 0.5 MBC (either 1 or 2 mg l^{-1}) gentamicin were used. At 0, 6 and 24 h, serial dilutions were plated onto blood agar and incubated overnight. A 100-fold difference in c.f.u. ml^{-1} between the combination of ampicillin and gentamicin and the most effective single antibiotic was used as the cut-off for synergy. For the combination to be considered synergistic against a given isolate, two out of three samples had to show synergy at a given time and antibiotic concentration.

In the isolate from our patient there was no synergy between ampicillin and gentamicin at any time point or ampicillin concentration (Fig. 1a). Neither did we see a synergistic bactericidal effect in the isolate from the patient with diverticulitis (Fig. 1b). In the two other isolates, from the patient with IE (Rasmussen *et al.*, 2014) and the child with bacteraemia with unknown focus, synergy was seen for the combination of 0.5 MBC ampicillin and gentamicin at both 6 and 24 h (Fig. 1c, d). No synergy was seen using 1 MBC ampicillin. Table 1 gives the log additional decrease in c.f.u.

MIC values were also determined using Etests (BioMérieux), either alone or in combination. The MIC was either 0.25 or 0.38 for ampicillin and between 1 and 2 for gentamicin (Table 1). To determine the fractional inhibitory concentration (FIC), the MIC on MIC method was employed (Pankey *et al.*, 2013), with the modification that MH-F plates were used. FIC was defined as (MIC for ampicillin in combination/MIC for ampicillin alone)+(MIC for gentamicin in combination/MIC for gentamicin alone). Recorded FIC values were 2 in three isolates and 1.2 in one isolate (Table 1). A FIC below 0.5 indicates synergy and thus there was no synergy between ampicillin and gentamicin in the inhibition of growth of the *L. garvieae* isolates.

Discussion

Here we describe a case of a potential IE caused by *L. garvieae*, which is an uncommon cause of invasive

Table 1. Antibiotic effects on isolates of *L. garvieae*

	MIC Amp	MIC Gen	FIC	MBC Amp	MBC Gen	Median additional killing with combination*				Bactericidal synergy
						0.5 mg l^{-1} Amp, 6 h	0.5 mg l^{-1} Amp, 24 h	1 mg l^{-1} Amp, 6 h	1 mg l^{-1} Amp, 24 h	
Case isolate	0.38	1.5	2	1	2	−0.11	0.44	−0.11	0.7	No
Isolate 1	0.25	2	2	1	4	0.65	2.6	0.49	1.4	Yes
Isolate 2	0.38	1.5	1.2	1	4	0.25	>2	>0	0.6	Yes
Isolate 3	0.38	1	2	1	2	1.3	−0.22	0.067	0.24	No

Amp, ampicillin; Gen, gentamicin.
*log additional decrease in c.f.u.

infections in humans. The mode of transmission and infection is not clear in this case as the patient had not been in contact with fish. The patient was treated with a combination of gentamicin and ampicillin and developed permanent vestibular damage presumably due to aminoglycoside toxicity. Therefore, we wanted to scrutinize the role of aminoglycosides in the treatment of lactococcal IE. Combination therapy employing a possible synergistic killing effect is widely used for IE caused by streptococci and enterococci. However, aminoglycosides are toxic, as illustrated by this case, and there are no clinical studies supporting the use of combination therapy. For IE caused by lactococci, many patients have been treated with aminoglycoside combinations (Rasmussen *et al.*, 2014; Rostagno *et al.*, 2013; Russo *et al.*, 2012) despite the fact that synergy has not even been demonstrated *in vitro*. Considering the basic principle of *primum non nocere*, potentially harmful treatments should be well-justified. For the causative isolate in the case described herein, there was no synergy between ampicillin and gentamicin, neither in the *in vitro* killing assay nor in the assay based on inhibition of bacterial growth, and therefore there are not even valid theoretical arguments for the use of aminoglycosides in this case. It should be noted that the analyses of the isolates were performed after the completion of the therapy in this patient and that beta-lactam and aminoglycoside combination has previously been employed in *L. garvieae* infections, including IE.

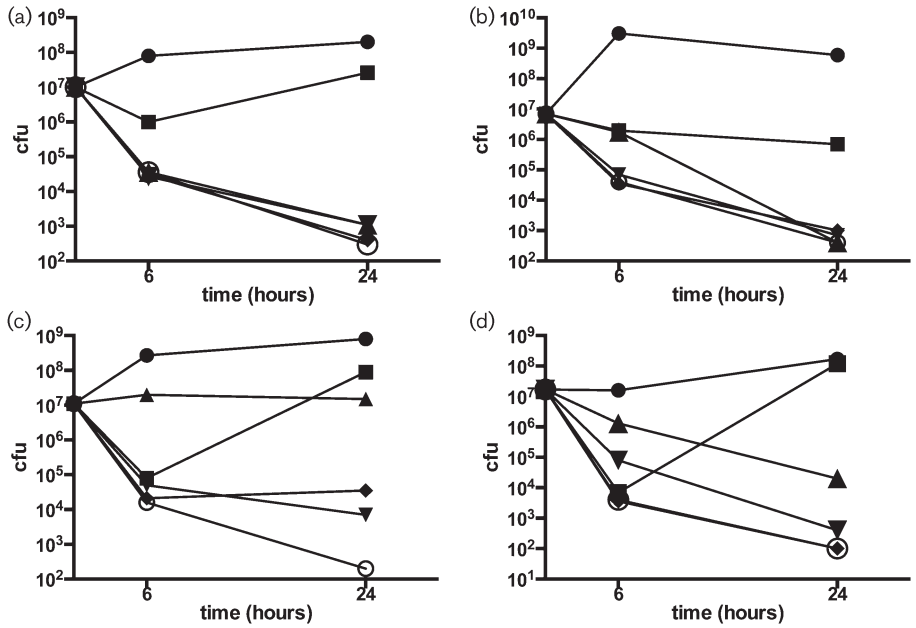

Fig. 1. Time-kill curves for different blood isolates of *L. garvieae* are shown. All experiments were performed three times and the median value is given. (a) Isolate from the patient described in this work, (b) from a patient with diverticulitis, (c) from a patient with IE, and (d) from a child with unknown focus of infection. Conditions used were: control (●), gentamicin (■), 0.5 MBC ampicillin (▲), 1 MBC ampicillin (▼), 0.5 MBC ampicillin and gentamicin (○) and 1 MBC ampicillin and gentamicin (♦).

For the four *L. garvieae* isolates tested here, synergy was noted for two isolates using the time-kill method and for none of the isolates using the Etest on Etest method. For the two isolates where no synergy was noted, the combination of ampicillin and gentamicin offered no increased killing at all. Of course it is difficult to draw conclusions from *in vitro* experiments and many aspects of the *in vivo* situation are not accounted for in our experimental setup. For example, concentrations of ampicillin were likely much higher in the patient described in this report than in the *in vitro* experiments. However, since *in vitro* evidence is the basis for the use of combination therapy, the lack of synergy in several *L. garvieae* isolates should call for restricted use of aminoglycosides in *L. garvieae* infections. The clinician must carefully weigh the risks of aminoglycoside toxicity against the *potential* benefit of using a treatment with a *potential* bactericidal synergy *in vitro*. Unfortunately, the decision must be based on empirical grounds since the Etest on Etest method, which potentially could be used in routine laboratories, does not provide relevant information.

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