Resistance to β-lactam antibiotics in *Bacteroides* spp.

R. EDWARDS

Division of Microbiology and Infectious Diseases and PHLS Laboratory, University Hospital, Queen’s Medical Centre, Nottingham NG7 2UH

*Bacteroides* spp., particularly *B. fragilis*, are well-recognised bacterial pathogens. Production of the typical β-lactamases of *Bacteroides* restricts the therapeutic use of β-lactam agents mainly to the β-lactamase inhibitor combinations and carbapenems. These compounds have the advantage of broad-spectrum activity and the ability to combat polymicrobial infections. Resistance of *Bacteroides* spp. to β-lactam antibiotics appears to be increasing, largely because of an overall increase in β-lactamase activity. There has been a rise in the prevalence of isolates showing high-level production of typical *Bacteroides* β-lactamases and an increase in reports other potent β-lactamase types. In the case of *B. fragilis*, metallo-enzymes are a particular threat to current therapeutic practice, as they are not inhibited by common β-lactamase inhibitors and are able to hydrolyse carbapenems. The presence of permeability barriers may confer low-level β-lactam resistance and supplement the effect of β-lactamase activity. There are also sporadic reports of loss of β-lactam activity because of reduced affinity of the penicillin-binding proteins.

Introduction

The pathogenic potential of gram-negative, non-sporulating, anaerobic bacilli has been recognised since the late 19th century [1]. Infections caused by these organisms are now known to be common and can sometimes be life-threatening. *Bacteroides* spp. (members of the former *B. fragilis* group) are the anaerobes encountered most frequently in clinical specimens. They are recognised as being among the most resistant of the anaerobes to antimicrobial agents, including β-lactam antibiotics, and it is of concern that the number of isolates that display resistance is increasing [2, 3].

This review focuses on the importance of *Bacteroides* spp. in human disease and the role of β-lactam compounds in treating these infections. The degree of resistance of clinical *Bacteroides* isolates to β-lactam antibiotics is discussed and the most effective β-lactam agents are high-lighted. Mechanisms of resistance to these compounds, including antibiotic degradation, reduced cell wall permeability, and lack of antibiotic binding to lethal target sites, are examined.

The role of *Bacteroides* spp. in infection

Anaerobic bacteria involved in infections are commonly derived from complex normal flora and, therefore, are found usually in association with other bacterial types. The major reservoir of bacteroides in the human body is the colon. The numerically dominant *Bacteroides* spp. in the normal colonic flora are *B. distasonis*, *B. vulgatus* and *B. thetaiotaomicron*, with only 5% of the cultivable colonic bacteria comprising *B. fragilis* [4], although this figure has been challenged as being an under-estimate [5]. *B. fragilis*, because of its unique virulence properties, is associated more commonly with infections than are the other *Bacteroides* spp. It is the most important pathogen among anaerobic bacteria, accounting for c. 25% of all anaerobic bacteria isolated from clinical specimens [3].

The most common infections with *Bacteroides* spp. in general, and *B. fragilis* in particular, are intra-abdominal abscesses, peritonitis and wound infections associated with the large intestine [6]. *Bacteroides* spp. are also principal pathogens of the female genital tract and pelvic abscesses [7, 8], and are implicated in anaerobic pulmonary infections [3, 9]. *B. fragilis* is implicated frequently in brain abscesses secondary to otitis media [10], and is a major pathogen in cases of
diabetic foot ulcer [3, 11]. Bacteraemia is commonly associated with *B. fragilis* infections, but endocarditis caused by *Bacteroides* spp. is rare [12].

**Susceptibility of *Bacteroides* spp. to β-lactam antibiotics**

Resistance of a wide range of bacterial pathogens to antimicrobial agents is a growing problem, and includes the reduced effectiveness of β-lactam compounds against *Bacteroides* spp. [13]. *Bacteroides* spp. are, almost invariably, relatively susceptible to benzylpenicillin; this is in contrast to most other clinically important anaerobic bacteria, including *Prevotella* spp. and *Porphyromonas* spp. [14]. The other penicillins and the cephalosporins (excluding the related cephalexins and oxa-cephems) generally also show low activity towards *Bacteroides* spp. [15–17]. However, bacteroides are more susceptible to some of the acylureidopenicillins, such as piperacillin [18]. Full susceptibility to benzylpenicillin and other β-lactam agents can normally be restored by addition of the β-lactamase inhibitors clavulanic acid, sulbactam and tazobactam [19].

The cephamycins are more active than conventional cephalosporins against *Bacteroides* spp. [18]. Low-level resistance to cefoxitin (MIC of 32 mg/L) was recognised in bacteroides immediately after the introduction of this antibiotic in the late 1970s [20]; high-level resistance (MIC of ≥ 64 mg/L) was first documented in 1983 [21]. However, resistance is uncommon; in a European study published in 1992, 3% of *Bacteroides* isolates were resistant to cefoxitin at a break point of 32 mg/L [22]. *Bacteroides* spp. are also susceptible to the oxa-cephem latamoxef, which exhibits greater in-vitro activity than cefoxitin [18]. In general, *B. fragilis* is more susceptible to these antibiotics than other *Bacteroides* spp. [23, 24].

Potent activity against *Bacteroides* spp. is displayed by the carbapenems, with MIC50s of imipenem and meropenem of 0.06–0.12 mg/L [25, 26]; comparable activity has been shown with the recently developed compound biapenem [27]. Carbapenem resistance is low, with < 2% of *Bacteroides* isolates reported as resistant [22, 28, 29], while < 10% of isolates show reduced susceptibility (imipenem MIC of 1–4 mg/L) [30]. The carbapenems, together with the clavulanate-potentiated penicillins, are the most active β-lactam compounds against *Bacteroides* spp. [18, 19, 22, 28, 29].

**β-Lactam antibiotics in the treatment of *Bacteroides* infections**

As most infections involving *Bacteroides* spp. occur in combination with aerobic pathogens, antibiotic therapy must be directed at both the anaerobic and aerobic components. In these situations, metronidazole is commonly used in combination with an anti-aerobe antibiotic, normally a cephalosporin or an aminoglycoside. Another possible option for prophylaxis and the treatment of bacteroides infections is the use of single broad-spectrum compounds. These would be simpler to administer and may be preferable financially. Candidates for such antibiotics include the carbapenems and cephapemycins, although the latter, as mentioned previously, are intrinsically less active. The combinations of penicillins with β-lactamase inhibitors are also useful therapeutic preparations to combat these infections.

**Mechanisms of resistance to β-lactam antibiotics**

To inhibit gram-negative bacteria, β-lactam antibiotics must be able to penetrate the outer membrane, pass through the periplasmic space, and arrive with undiminished potency at the penicillin-binding proteins (PBPs) in the cytoplasmic membrane. Co-valent binding with the PBPs has then to occur for the lethal affect to take place. Mechanisms of resistance that interfere with this chain of events are: blockage of transport of the agent into the cell; inactivation of the antibiotic, usually within the periplasmic space; and alteration of target sites.

**Reduced antibiotic penetration**

The ability of an antibiotic to reach its site of action is a prerequisite for drug action. In gram-negative bacteria, the outer membrane presents a physical barrier to the penetration of antibiotics into the periplasmic space. The outer-membrane protein (OMP) composition of *Bacteroides* spp. is complex [31, 32] and, until recently, no specific porin molecules had been identified in bacteroides. However, since 1992, studies with liposome assays have revealed pore-forming ability of fractions isolated from the outer membranes of strains of *B. distasonis* and *B. fragilis* [33–35].

The ability of β-lactam compounds to permeate the outer membrane of gram-negative bacteria is dependent on the physicochemical properties of the antibiotic [36]. With *B. fragilis*, the rank order of permeative ability of selected β-lactam antibiotics was reported by Cuchural et al. [37], with cephaloridine the most rapid, followed by imipenem, cefotaxime, cefoxitin, cephalothin and latamoxef. Investigation of factors influencing permeability yielded results similar to those described for *Escherichia coli*, in which increases in negative charge and molecular mass were associated with decreased antibiotic uptake [36]. However, unlike the situation in *E. coli*, increased drug hydrophobicity was associated with increased uptake by *B. fragilis*. It has been proposed that the different effects of hydrophobicity on permeability are caused by dissimilarites in the lipopolysaccharide...
The role of permeability barriers in β-lactam resistance in Bacteroides spp. has been observed indirectly. Dornbusch et al. [40] examined strains of B. fragilis with decreased susceptibility to cefoxitin and found no correlation between β-lactamase production and resistance to cephamycins. It was postulated that changes in the cell wall, causing decreased penetration of the antibiotic, could be a resistance factor.

Results from crypticity measurements (the ratio of β-lactamase activity of broken cells to the activity of intact cells), with cephaloridine as substrate, indicate that limited outer-membrane permeability to β-lactam antibiotics contributes to resistance in certain strains of B. fragilis [41]. Also, β-lactamase-mediated imipenem resistance in B. fragilis has been shown to be associated with a barrier to drug permeation, as determined by crypticity measurements [42]. In contrast, a recent study showed no correlation between the levels of imipenem resistance of B. fragilis isolates and crypticity or other permeability measurements that take into account kinetic factors involved in drug permeation (personal unpublished results).

EDTA has been used to increase the permeability of the cell wall in an attempt to assess the importance of impermeability of this wall in terms of antibiotic resistance [43]. The addition of EDTA enhanced the activity of cefoperazone significantly against β-lactamase-negative, as opposed to β-lactamase-positive, bacteria, indicating that cell impermeability was a major mechanism of resistance to cefoperazone in β-lactamase-negative isolates. Also, an increase in the susceptibility of bacteroides to cephalosporins in the presence of EDTA has been observed, suggesting a role for a permeability barrier in addition to β-lactamase, with the exception of B. distasonis, which showed only a permeability barrier [44].

Evidence of an association between cefoxitin resistance and altered porin proteins in Bacteroides spp. was put forward by Piddock and Wise [45]. They described two cefoxitin-resistant strains, one of which was B. fragilis and the other B. thetaiotaomicron, in which resistance was not β-lactamase mediated, but related to alterations in OMP profiles together with PBP changes. An OMP, possibly a porin protein, of c. 50 kDa was absent from both strains. Other studies have shown conflicting evidence of the association between reduced susceptibility to carbapenems and reduced expression or loss of OMPs [39, 46].

β-Lactamases

The most important mechanism of resistance to β-lactam antibiotics in Bacteroides spp. is β-lactamase-mediated hydrolysis of the β-lactam ring, which renders the antibiotic inactive. Production of elevated amounts of β-lactamase by Bacteroides strains is associated with high-level resistance to β-lactam antibiotics [47].

The first report of the penicillin-destroying activity of B. fragilis was by Garrod in 1955 [48], who showed that two of 31 strains examined were able to destroy penicillin in solution. The production of β-lactamases by 14 penicillin-resistant strains in a collection of 29 isolates of B. fragilis was documented in 1968 [49]. It is now recognised that most Bacteroides strains (c. 90%) produce small amounts of a constitutive β-lactamase [19, 50]. B. distasonis isolates are associated with the least amount of β-lactamase production, with 58–80% of strains displaying β-lactamase activity [14, 51].

The proportion of isolates of Bacteroides spp. that are capable of high-level β-lactamase production appears to be increasing. In 1977, Olsson et al. [50] reported that 6% of B. fragilis isolates produced elevated amounts of β-lactamase. Ten years later, 25% of B. fragilis group isolates from the USA produced high levels of β-lactamase [52]. In Nottingham, the percentage of clinical Bacteroides isolates displaying raised β-lactamase production increased from 17% to 25% between 1986 and 1995 [30]. In those cases in which elevated levels of typical β-lactamases are found, insertion elements may be responsible for increased expression of the cepA gene that controls production of these enzymes [53].

The first detailed characterisation of a β-lactamase produced by a strain of B. fragilis exhibiting high-level ampicillin resistance was provided by Anderson and Sykes [54]. The enzyme was shown to be a cephalosporinase rather than a penicillinase, it was not inducible, and its production correlated with high-level resistance to β-lactam antibiotics. Further characterisation of the common β-lactamases from B. fragilis has shown them to have a molecular mass of 30–40 kDa, and to be inhibited by cloxacillin, pCMB and clavulanic acid [55–57]. Cefoxitin, latamoxef and imipenem are typically resistant to hydrolysis by these enzymes [58]. β-Lactamases from B. fragilis, B. thetaiotaomicron, B. vulgatus and B. uniformis show species-specific differences in terms of substrate profile and pl values that range between 4.9 for B. fragilis and 4.25 for B. thetaiotaomicron [59]. It is now recognised that most Bacteroides isolates produce at least one β-lactamase with activity that is species-specific, although they share the general characteristics described previously [47, 60].

These properties differ from those of β-lactamases from aerobic bacteria. However, Bacteroides β-lactamases do bear some resemblance to class I enzymes of the Richmond and Sykes scheme, except in terms
of inhibition by pCMB and clavulanic acid [59, 61]; an additional class (VI) of this scheme was put forward by Neu [62] to accommodate β-lactamases from Bacteroides spp. In the most recent differential scheme developed by Bush, Jacoby and Medeiros, typical β-lactamases produced by B. fragilis have been placed in group 2e [63]. Rogers et al. [64] characterised the cephalosporinase gene cepA from B. fragilis that exhibits high specific activity and showed that these typical β-lactamases form a distinct subgroup of Ambler molecular class A [65]. Similarly, a clavulanate-sensitive cephalosporinase produced by B. vulgatus, which was capable of slow degradation of cefoxitin, appears to belong to a new class-A homology group [66]. Analysis of β-lactamase from B. uniformis revealed a species-specific Bush group 2e enzyme that belongs to Ambler class A [67]. However, an unusual β-lactamase from B. uniformis strain B 371, which appears to belong to group 2e, has been reported. This β-lactamase showed a substrate profile that resembles enzymes of Ambler class C, except for its inhibition by clavulanic acid [68]; class C enzymes have not been encountered previously in Bacteroides spp.

Other types of β-lactamase, apart from the common group 2e enzymes, have been reported in bacteroides. Sato et al. [69] described a B. fragilis β-lactamase that was a potent penicillinase, had weak cephalosporinase activity, was inhibited by pCMB and had an isoelectric point of 6.9. This enzyme has been designated a member of Bush group 2d [63]. Also, atypical β-lactamases from strains of Bacteroides spp., such as B. fragilis strain TAL4170 and B. uniformis strain 2986, have been reported that inactivate cefoxitin, show diverse pl values and exhibit various levels of susceptibility to clavulanic acid [20, 21].

A distinct class of B. fragilis β-lactamase that inactivated a wide range of β-lactam substrates usually considered stable to hydrolysis, including cephamycins and carbapenems, was reported in 1986 by Cuchural et al. [42]. These enzymes were inhibited by the ion chelator EDTA, and zinc ions completely reversed this inhibition. Clavulanic acid and sulbactam did not inhibit activity of these β-lactamases [70]. Metallo-β-lactamases from other B. fragilis strains with similar properties have been described subsequently [71, 72]. Hedberg et al. [73] also characterised an imipenem-hydrolysing B. fragilis metallo-β-lactamase, albeit with a substrate profile that differed from those described previously [72] while other characteristics such as inhibition profiles and physical properties were similar. All these enzymes caused substantial resistance to imipenem (MICs of >100 µg/mL), had pl values in the range 4.5–5.2 and a molecular mass of 25–33 kDa when determined by SDS-PAGE [74]. These metallo-β-lactamases belong to Ambler’s molecular class B and the Bush functional group 3 [63, 70], and are similar to those produced by other bacterial species such as Stenotrophomonas maltophilia and Aeromonas hydrophila [75]. Other clinical isolates of B. fragilis have been described that produce EDTA-sensitive, clavulanic acid-resistant carbapenemases, although these showed lower levels of resistance to imipenem, ranging from reduced susceptibility to intermediate resistance (MICs of 0.25–32 µg/mL) [76, 77].

Gene sequencing and DNA probes have confirmed the presence of highly homologous cfrA or cfrA genes in clinical isolates of B. fragilis from the USA, UK and France that produce metallo-β-lactamase [78–81]. DNA sequence analysis has shown that metallo-β-lactamases produced by three B. fragilis strains, for which the MICs of imipenem varied over a 16-fold range, differed at five amino-acid residues [82]. These enzymes were over-expressed, purified and their kinetic values for a variety of β-lactam antibiotics determined. By these means, it was found that the five amino-acid substitutions affected the hydrolysing activity of these β-lactamases only modestly and that the differences in susceptibilities were a reflection of the level of gene expression. High-level resistance among strains harbouring the cfrA gene is, therefore, a consequence of enhanced metallo-β-lactamase production rather than enzyme differences or permeability factors [82] (personal unpublished results).

Podglajen et al. [83] showed with a DNA probe that 2.2% of the B. fragilis clinical isolates studied carried the cfrA gene. In two-thirds of these, the cfrA genes were ‘silent’ and were associated with strains of reduced susceptibility (imipenem MICs of ≤2 µg/mL). Selection of imipenem resistance by single step mutation, associated with zinc β-lactamases, has been detected in B. fragilis isolates that appeared initially to be moderately susceptible to imipenem [81]. Expression and resistance rely on the presence of an insertion sequence upstream of the cfrA gene, and this arrangement can occur spontaneously at a frequency of c. 10^{-7} [81, 84]. The implications of these observations are disturbing. It would seem that B. fragilis strains that possess the ‘silent’ cfrA gene, and appear susceptible to carbapenems, have the potential to convert to high-level β-lactam resistance, including resistance to carbapenems. Indeed, this situation has been observed recently in vivo, with a B. fragilis strain developing resistance during treatment with imipenem of a patient in Nottingham [85]. Paradoxically, we have been unable to generate resistant mutants in vitro from this or other strains that appeared likely candidates for such conversion.

The occurrence of metallo-β-lactamases in isolates of B. fragilis pre-dates the widespread use of carbapenems [86]. These broad-spectrum enzymes may have provided protection from common β-lactam antibiotics used before 1987, although they hydrolyse cefoxitin less efficiently than carbapenems. Interestingly, B. fragilis is the only Bacteroides spp. in which metallo-
β-lactamases have so far been reported. Several attempts in Nottingham have failed to detect these enzymes in other gram-negative anaerobes. Strains carrying the cfxA gene have been separated by molecular typing into a distinct genotype of *B. fragilis* with a particular OMP profile [39, 87].

Other types of imipenemases produced by *Bacteroides* spp. have been described. In 1983, Yotsuji *et al.* [88] reported a potent β-lactamase produced by a *B. fragilis* strain with a similar substrate profile to that described by Cuchural *et al.* [42], i.e., capable of hydrolysing cephamycin derivatives and imipenem, and not susceptible to clavulanic acid. However, EDTA inhibition was not mentioned in this report and this enzyme has been assigned to group 4 of the Bush classification scheme [63]. Also, imipenem resistance (MIC of 16 mg/L) in a *B. distasonis* isolate has been attributed to the combination of production of an unusual imipenem-inactivating serine-β-lactamase and impermeability [89]. This enzyme, with a molecular mass of 160 kDa, was inhibited by clavulanic acid and sulbactam, but not by EDTA.

Further attempts have been made to classify the wide variety of β-lactamases encountered in bacteroides [76, 77]. β-Lactamases produced in raised amounts by clinical *Bacteroides* isolates have been characterised according to their antibiotic degradation, inhibitor profiles and specific activity. Two types equated with the typical β-lactamases of *Bush group 2e* and the metallo-β-lactamases of group 3. A third type was produced by strains that exhibited resistance to benzylpenicillin and cefoxitin, and reduced susceptibility to imipenem; they also displayed intermediate or high specific activity. These enzymes showed similar inhibition profiles and hydrolysed β-lactamase-stable compounds other than imipenem. Others showed reduced susceptibility to cefotaxim and imipenem, but these enzymes were unable to hydrolyse these antibiotics.

β-Lactamase production in bacteroides has occasionally been found to be transferable. Most genes for β-lactamase production in bacteroides are located on the chromosome, but the penicillinase belonging to group 2d has been transferred from a *B. fragilis* strain to susceptible strains of *B. fragilis* and *B. vulgatus* by in vitro filter mating, and this transfer was considered to be plasmid-mediated [69]. Transmission of resistance to cefoxitin has also been reported. *B. fragilis* strain TAL4170 was shown to transfer β-lactamase-mediated cefoxitin resistance to a susceptible *B. fragilis* recipient by conjugation [90]. Also, cefoxitin resistance transfer has been demonstrated in a strain of *B. thetaiotaomicron*, although the precise resistance mechanism for this isolate was not determined [91]. Although genes coding for metallo-β-lactamases of *B. fragilis* have been shown to be present on the chromosome [79], Bandoh *et al.* [92] reported a strain of *B. fragilis* that contained a metallo-β-lactamase gene on a small plasmid transferable by conjugation. This situation increases greatly the potential for spread of the enzyme.

**Altered PBPs**

In many species of aerobic gram-negative bacteria, modified PBP affinity has been shown to result in resistance to β-lactam antibiotics [93]. However, there is less evidence of changes in particular PBPs as a resistance mechanism in *Bacteroides* spp. Bacteroides are naturally resistant to some β-lactam antibiotics, including monobactams and temocillin, because of the poor affinity of these compounds for the PBPs; aztreonam binds poorly or undetectably to all PBPs of *B. fragilis* [94].

Accounts of the number and molecular mass of PBPs of *Bacteroides* spp. are conflicting, although three high molecular mass PBPs are found consistently and others of lower molecular mass are seen sporadically [95–99]. Analysis of PBPs from fully sensitive *B. fragilis* strains in our laboratory yielded results broadly similar to those of Wexler and Halebian [96] and Yotsuji *et al.* [99]; three PBPs of 91, 80 and 69 kDa were universal, while two others of 63 and 47 kDa were detected occasionally [100]. The PBPs of *Bacteroides* spp. differ from those of *E. coli* in terms of their affinity for β-lactam antibiotics and in the morphological consequences of inhibition of these proteins. The primary target in bacteroides for most β-lactam antibiotics is PBP 2, which is involved in septation and corresponds to PBP 3 of *E. coli*. The PBP 1 complex is usually the secondary target site and is associated with cell elongation, corresponding to PBP 1 of *E. coli*. Compounds such as imipenem and meropenem bind initially to PBP 3, causing round cells, and then to PBP 2. PBP 3 in bacteroides is, therefore, equivalent to PBP 2 in *E. coli* and is involved in cell shape. Imipenem also binds to PBP 1 at concentrations correlating with the MIC [95, 98].

Several workers have reported an association between reduced affinity of β-lactam compounds for the PBPs of *Bacteroides* spp. and resistance. Georgopapadakou *et al.* [98] observed reduced affinity of piperacillin, cefoperazone, cefotaxime, cefazidime and imipenem for PBP 2 in a resistant strain of *B. fragilis*. Changes in the affinity of PBP 1 or PBP 2 in laboratory-derived mutants have also been correlated with a decrease in susceptibility to cefotaxim [45]. Decreased affinity for PBP 3, and not β-lactamase hydrolysis or membrane permeation, has been implicated as the important factor in the resistance to ceftizole, ceftazolin and cephalothin of a *B. fragilis* strain [99]. Also, the affinity of piperacillin for PBP 1 was reduced in a resistant strain of *B. uniformis*, as was the binding of cephalothin and cephaloridine to PBP 4 [101].
Wexler and Halebian [96] reported changes in both the PBP 1 complex and the affinity of one of the PBP 1 proteins for cefoxitin between cefoxitin-sensitive and cefoxitin-resistant strains of B. thetaiotaomicron. Resistant stains of B. uniformis also showed changes in the PBP 1 complex in comparison with sensitive strains. Furthermore, a laboratory-derived cefoxitin-resistant mutant of B. distasonis displayed reduced binding to the PBP 1 complex compared with its wild-type parent and cefoxitin-sensitive revertant. No obvious changes in OMP profiles were detected that might indicate changes in permeability. In a recent study of B. fragilis strains that do not produce carbapenemase, resistance to imipenem was not associated with loss of high molecular mass PBPs, but with reduced binding of imipenem to these PBPs, together with the appearance of a new low molecular mass PBP [100].

Concluding remarks

In common with other bacterial genera, resistance among the bacteroides will continue to increase with antibiotic use. The activity of β-lactam compounds is particularly under threat, as most strains possess β-lactamases and increased production of these enzymes enhances resistance to some commonly used agents. The potential for wider distribution of other potent β-lactamases, including the metallo-β-lactamases, is also worrying. The possibility of conversion of the metallo-β-lactamase gene from ‘silent’ to full expression, with corresponding resistance to carbapenem and common β-lactamase inhibitor combinations, is of particular concern. There is a need to monitor the degree of resistance among clinical isolates of Bacteroides spp., together with β-lactamase types and levels of production. Also, prudent use of highly active antibiotics is necessary to extend their useful life.

References

33. Wexler HM, Bailey LC, Fisher G. The isolation and characterization of a major outer-membrane protein from


44. Malouin F, Lamothe F. The role of β-lactamases and the permeability barrier on the activity of cephalosporins against members of the Bacteroides fragilis group. Can J Microbiol 1987; 33: 262–266.


