negative staphylococci are readily inhibited by amoxicillin–clavulanic acid (Bush, 1988; Goldstein and Caitron, 1988). Minimum lethal concentrations (MICs) are slightly higher than for penicillin-sensitive *S. aureus* (Fuchs *et al.,* 1983; Slocombe *et al.,* 1984). Amoxicillin–clavulanic acid shows greater *in vitro* activity than flucloxacillin against many beta-lactamase-producing strains; this *in vitro* advantage does not occur with *S. aureus* strains that produce large amounts of beta-lactamase (Thomas *et al.,* 1985). Staphylococcal beta-lactamases are molecular class A (Bush group 2a) and are further subdivided, initially serologically, into subtypes A to D (Richmond, 1965; Livermore, 1995). The epidemiological distribution of these varies: Types A and C are most common and type D is rare (Livermore, 1995). Organisms with type C beta–lactamases are less susceptible to amoxicillin–clavulanic acid and to other beta-lactamase inhibitors such as piperacillin–tazobactam (Bonfiglio and Livermore, 1994; see Chapter 17, Piperacillin/tazobactam). Methicillin-resistant *S. aureus* and coagulase-negative staphylococci are resistant to amoxicillin–clavulanic acid (Granger *et al.,* 1989) because resistance is mediated by an altered penicillin-binding protein (PBP) 2a encoded by the *mecA* gene. Recently described methicillin-resistant. *aureus* (MRSA) isolates with PBP2c encoded by *mecC* have beta-lactam resistance mediated by a combination of PBP2c and a closely linked beta-lactamase ($BlaZ_{LGA251}$), the latter of which is required for resistance (Ba *et al.,* 2015). Penicillin and clavulanic acid combinations, including amoxicillin–clavulanic acid, have *in-vitro* and *in-vivo* activity against these *mecC*-containing MRSA. Furthermore, Mehta *et al.* (2012) reported that beta-lactams, including amoxicillin–clavulanic acid, had synergistic activity with daptomycin against daptomycin-resistant MRSA strains and, in addition, prevented the selection of resistance to daptomycin.

Beta-lactamase-producing strains of *E. faecalis* are sensitive to amoxicillin–clavulanic acid, provided this is the only resistance mechanism (Ingerman *et al.,* 1987). However, these strains are uncommon, whereas the widespread beta-lactam resistance in *E. faecium* relates to production of PBP5 and therefore causes amoxicillin–clavulanic acid resistance.

## GRAM-NEGATIVE AEROBIC BACTERIA

Beta-lactamase-producing strains of *Neisseria gonorrhoea, H. influenzae, H. ducreyi,* and *M. catarrhalis* are susceptible to amoxicillin–clavulanic acid (Girouard *et al.,* 1981; Farmer and Reading 1982; Alvarez *et al.,* 1985; Dangor *et al.,* 1988; Cooper *et al.,* 1990). Strains of *N. gonorrhoea* and *H. influenzae* that are intrinsically resistant to penicillin G and amoxicillin are amoxicillin–clavulanic acid resistant (Powell *et al.,* 1991). Beta-lactamase-producing gonococcal strains that possess a 3.2 megadalton beta-lactamase plasmid are more sensitive to amoxicillin–clavulanic acid than strains possessing a 2.9, 3.05, or 4.4 megadalton plasmid (Rice and Knapp, 1994). *N. meningitidis* remains highly susceptible to amoxicillin. Anta *et al. (*2002) observed that clavulanic acid at subinhibitory concentrations enhanced the *in vitro* bactericidal activity of amoxicillin against *N. meningitidis* and suggested amoxicillin–clavulanic acid could have a potential role in nasopharyngeal eradication of the organism.

Amoxicillin–clavulanic acid inhibits many *Enterobacteriaceae* that produce beta-lactamases associated with amoxicillin resistance. Thus amoxicillin-resistant *E. coli, Klebsiella pneumoniae, Proteus mirabilis,* some *Citrobacter* spp., and, to a lesser extent, *Yersinia enterocolitica* are often amoxicillin–clavulanic acid sensitive (Gaspar and Soriano, 1981; Fuchs *et al.,* 1983; Slocombe *et al.,* 1984; Bush, 1988; Roy *et al.,* 1989; Kahlmeter, 2003). However, some strains of *E. coli* hyperproduce TEM-1 beta-lactamase; these may need higher concentrations of clavulanic acid to inhibit the enzyme and may be resistant to the standard amoxicillin–clavulanic acid formulation (Wu *et al.,* 1994). Beta-lactamase-producing *Salmonella* and *Shigella* spp. are readily inhibited by amoxicillin–clavulanic acid (Neu and Fu, 1978). *Enterobacter* spp., *C. freundii, Serratia* spp., *Morganella morganii, Providencia stuartii*, and other AmpC beta-lactamase producers that are amoxicillin-resistant are also resistant to amoxicillin–clavulanic acid because clavulanic acid has minimal activity against these enzymes (Slocombe *et al.,* 1984; Weber and Sanders, 1990; Bush *et al.,* 1991; Livermore, 1995; Paterson, 2006). Clavulanic acid is in fact a weak inducer of these beta-lactamases, but this is not clinically significant as there is no role for amoxicillin–clavulanic acid in treatment of infections caused by these organisms (Livermore *et al.,* 1989; Rolinson, 1989; Bush *et al.,* 1991).

Susceptibility of *E. coli* to amoxicillin–clavulanic acid by macrodilution may depend on testing methodology, and the variations between EUCAST (which uses a fixed 2-mg/l clavulanic acid concentration and no intermediate category) and CLSI (which uses a 2:1 fixed ratio of a amoxicillin–clavulanic acid) have been recently described. Switching from the CLSI to the EUCAST recommendation was associated with an increase in *E. coli* amoxicillin–clavulanic acid resistance, from 19% in 2010 to 31% in 2011, but was not observed among laboratories that did not adopt EUCAST standards (Leverstein-van Hall *et al*., 2013). The change was attributed to switching to a fixed clavulanic acid concentration. Furthermore, testing by EUCAST-broth microdilution resulted in more isolates being labeled resistant than testing by disk diffusion or E test (using fixed ratios); preliminary data suggested clinical response was better correlated with fixed concentration than fixed ratios.

In a similar study Díez-Aguilar *et al.* (2015) found agreement between fixed concentration and fixed ratio microdilution in only 25.6% of *E. coli*. Discrepancies were particularly evident with ESBL-producing strains; 55% of isolates were resistant with EUCAST-fixed 2-mg/l clavulanic acid, whereas 90% were susceptible by CLSI breakpoint and fixed 2:1 ratio. The ESBL isolates were all susceptible using EUCAST urinary breakpoints for uncomplicated urinary tract infection; however, automated susceptibility methods do not commonly incorporate these concentrations (Díez-Aguilar *et al*., 2015). The results most predictive of clinical outcome remain to be clarified, but overreporting of resistance, particularly for urinary isolates, has the unintended consequences of increasing