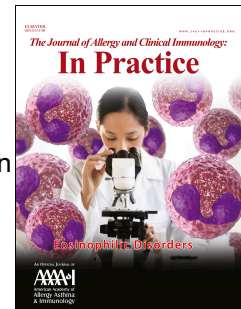


# Journal Pre-proof

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Minor Determinants of Penicillin and Amoxicillin are Still Key Components of Penicillin Skin Testing

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Background: The positive rate and pattern of penicillin skin testing (PST) has been reported to be decreasing and changing. Previous studies differ in which penicillin component is the dominant component in positive PST.

Objective: This study was conducted to characterize past and current PST patterns to determine if different determinants in PST have changed overtime.

Methods: A retrospective review of electronic medical records (January 2001-December 2017) was performed for patients who underwent PST. Data was divided into four cohorts to see if trends occurred overtime. The cohorts were divided as follows: Cohort 1:2001-2005, Cohort 2: 2006-2010, Cohort 3: 2011-2015, and Cohort 4: 2016-2017.

Results: 30,883 patients underwent PST with the following breakdowns per cohort: Cohort 1- 6,536, Cohort 2- 10,372, Cohort 3- 10,640, Cohort 4- 3,335. 329 patients (1.0%) had a positive PST ( $\geq 3 \times 3$  wheal) with 110 in Cohort 1, 130 in Cohort 2, 67 in Cohort 3, and 22 in Cohort 4. 170 patients (0.5%) were positive with a  $\geq 5 \times 5$  wheal: Cohort 1- 54, Cohort 2- 72, Cohort 3-34, Cohort 4- 10. When the positive PST rates (Cohort 2: 1.25%, Cohort 3: 0.6%, and Cohort 4: 0.6%) were compared to Cohort 1 (1.7%), there was a significant decrease in positive PST rates ( $p=0.0278$ ;  $<0.0001$ ;  $<0.0001$ , respectively). When Cohort 1 positive rate to benzylpenicillin polylysine among the positive PST ( $\geq 3 \times 3$  wheal) was compared to the other cohorts (Cohorts 2-4), the percent positive of benzylpenicillin polylysine in PST was 27% compared to 21% ( $p=0.38$ ); 34% ( $p=0.5$ ); 18% ( $p=0.6$ ), respectively. When the positive PST was defined as  $\geq 5 \times 5$  mm wheal, the positive rate for benzylpenicillin polylysine in PST increased overtime (Cohort 2: 22%  $p=0.8$ ; Cohort 3: 32%  $p=0.3$ ; Cohort 4: 40%,  $p=0.264$ ) compared to Cohort 1 (19%).

Conclusion: Positive PST rate is decreasing. We demonstrate that despite benzylpenicillin polylysine solely positive rates remaining relatively stable, the minor penicillin determinants and amoxicillin play an important role in PST and their adoption into standard protocol for routine PST should be considered.

### Highlights Box:

What is already known about this topic? PST is an important diagnostic tool in the evaluation of IgE (immunoglobulin E)-mediated penicillin allergy. The positive rate and pattern of PST has been reported to be decreasing and changing.

What does this article add to our knowledge? This data shows the changes in positive PST over time and suggests that using a combination of benzylpenicillin polylysine and minor determinants with amoxicillin in the evaluation of patients with history of penicillin allergy is needed.

How does this study impact current practice management guidelines? The determinants used in PST may need to be re-evaluated and include the minor determinants and amoxicillin as standard protocol.

Key Words: Drug Allergy; Allergic Reaction; Antibiotic; Penicillin; Penicillin Skin Testing; Benzyl-penicilloyl.

Abbreviations:

DRESS: Drug Rash with Eosinophilia and Systemic Symptoms

IgE: Immunoglobulin E

PST: penicillin skin test

**Introduction:**

Penicillin and cephalosporin antimicrobials are two classes of  $\beta$ -lactam antimicrobial agents that are commonly associated with causing IgE-mediated hypersensitivity reactions.<sup>1-3</sup> The prevalence of self-reported history of a penicillin allergy has been estimated to be 10%.<sup>4</sup> Many of these are not true IgE-mediated hypersensitivities and clinically significant penicillin hypersensitivity is <5% and possibly as low as 1%.<sup>5</sup> It has previously been reported by Macy et al<sup>6</sup> that PST rates are falling overtime. However, this finding has not been recreated at a different geographical location since 2007.

PST consists of both major and minor determinants to indicate a possible hypersensitivity reaction. The major determinant (benzylpenicillin polylysine) is given that term because it detects the greatest number of penicillin allergic patients when skin testing is administered. The minor determinants (penicilloate and penicillin G potassium) help increase the sensitivity of detecting penicillin allergy on skin testing.<sup>7</sup> The aminopenicillins, amoxicillin or ampicillin, are also agents that can be used in PST. Amoxicillin self-reported allergy is increasing and has been reported to be as high as 65% of patients with penicillin allergy.<sup>16</sup> There are several studies that have included amoxicillin skin testing in PST. It has been reported that up to 45% of patients that test positive on PST tested positive solely to the aminopenicillins.<sup>8-10,16</sup> The minor determinants and amoxicillin are not standardized on PST in the United States. The only skin test agent that is currently FDA approved is penicilloyl polylysine. However, there is recent literature studying the use of a Penicillin Skin Test Kit that includes both minor determinant mixtures as well as amoxicillin using FDA standards.<sup>24</sup> Amoxicillin is commonly used currently as a challenge material after skin testing is negative with penicilloyl polylysine and penicillin G.<sup>11</sup> There has been no recent data to assess whether benzylpenicillin polylysine is currently the most

commonly found determinant to be positive on PST. Hence, we conducted this study to determine whether the positive rates of the various PST components are changing in frequency, to help determine the key components of the PST, and whether the overall rate of PST is changing in a large US medical center.

**Methods:***Study Population*

This study was conducted as a retrospective analysis of the cohort of patients who underwent PST at Mayo Clinic Rochester in the years 2001-2017 as captured in the electronic medical record. Data software retrieval was utilized to scan the electronic medical record and identify patients that underwent previous PST to create a database. PST occurred in both the inpatient and outpatient setting in patients specifically referred for reported penicillin allergy. A total of 30,883 PSTs were performed over this time frame. An additional 1244 patients underwent PST. However, they had a non-reactive histamine control and were excluded from the study population. Patients were excluded from PST if the previously described reaction to  $\beta$ -lactam antibiotics included severe non IgE-mediated drug hypersensitivity reactions such as major and minor erythema multiform, DRESS syndrome (Drug Rash with Eosinophilia and Systemic Symptoms), serum sickness, Steven-Johnson's syndrome, Toxic epidermal necrolysis, hemolytic anemia, etc. This study was approved by our institutional review board. Data was divided into four cohorts to see if trends occurred overtime. The cohorts were divided as follows: Cohort 1: 2001-2005, Cohort 2: 2006-2010, Cohort 3: 2011-2015, and Cohort 4: 2016-2017.

*Penicillin Skin Testing*

PST was conducted by using benzylpenicilloyl polylysine (Pre-Pen®), penicillin G potassium (Pfizerpen®), and penicilloate, as previously reported.<sup>17,18</sup> Commercially available Pre-Pen was unavailable between the years of 2004-2010, and an institutionally derived benzylpenicilloyl polylysine was used for PST. The penicilloate was produced by reacting penicillin G with 1N NaOH at a pH of 11.5 for 90 minutes, after which the pH was adjusted to 7.4 by addition of 1N HCl. The penicilloate was lyophilized and stored at 4° C. The penicilloate was diluted with

phosphate-buffered saline to 0.01 mol/L and filtered through a 0.22- $\mu$ m membrane for sterility weekly to be used for PST. The aqueous penicilloate was stored at 4° C. The benzylpenicilloyl polylysine was used according to the manufacturer's instructions, and the penicillin G potassium was used in a concentration of 6000 U/mL in phosphate-buffered saline. Amoxicillin was tested at a concentration of 4 mg/mL. Penicillin G potassium and amoxicillin are prepared weekly and stored at 4° C for skin testing. Histamine at 0.05 mg/mL was used as the positive control, and the negative control was phosphate-buffered saline.

Prick skin tests were performed on the volar surface of the forearm with each penicillin and control reagents. The skin test sites were examined at 15 minutes. A positive test result was defined as a wheal of 3x3 mm or greater with a surrounding zone of erythema above the negative control. Patients with negative prick test results to penicillin underwent intradermal testing. Intradermal skin tests were performed on the volar surface of the forearm. The test reagents were injected intradermally to produce an initial wheal of 2x2 mm. The skin test sites were examined at 15 minutes. A positive intradermal test was defined as a wheal of 3x3 mm or greater with a surrounding zone of erythema above the negative control. Patients with a wheal but without a flare on the PCN skin test (skin prick and intradermal) were considered to be equivocal and were included as a negative result.

#### *Statistical Analysis*

All demographic data was summarized using mean  $\pm$  standard deviation for age and gender with absolute number and percent. The overall positive PST and different components of the skin test (benzylpenicilloyl polylysine, penicillin G potassium, penicilloate, and amoxicillin) among the 4 different cohorts were summarized by absolute numbers and percent. Multiple penicillin

components could be positive on PST and therefore this is reflected in our dataset by not having all of the percent positives add up to 100 percent. A positive PST and the different components of the PST were defined as a wheal of  $\geq 3 \times 3$  mm or  $\geq 5 \times 5$  mm greater with a surrounding zone of erythema above the negative control. Both a positive wheal of  $\geq 3 \times 3$  mm or  $\geq 5 \times 5$  mm were used to account for different definitions of a positive PST.<sup>19, 20</sup>

Cohort 1 was used as the reference population to compare the different cohorts (2-4). Using the  $\chi^2$  test or Fisher's exact test, we compared the differences in the proportion of study cohort 1 and cohorts 2 to 4 to the positive PST and the different PST components. P value  $\leq 0.05$  was considered statistically significant.

**Results:**

A total of 30,883 patients underwent PST from January 2001-December 2017. 29,354 patients underwent outpatient PST testing and 1,529 patients underwent inpatient PST. 20,658 patients were female and 10,225 patients were male. 1,325 of these patients were less than 18 and part of our pediatric population. 48 patients tested positive in the pediatric population (3.6%). Using a  $\geq 3 \times 3$  wheal, 329 patients (1%) had a positive PST. When using a  $\geq 5 \times 5$  wheal to determine PST positivity, 170 patients (0.5%) had positive results. Of the 1,529 patients undergoing inpatient PST, 29 patients (1.9%) had a positive PST with a  $\geq 3 \times 3$  wheal and 15 patients (1%) had a positive PST with a  $\geq 5 \times 5$  wheal. 300 patients (1%) out of 29,354 patients tested in the outpatient setting had a positive PST  $\geq 3 \times 3$  wheal and 155 patients (0.5%) with a  $\geq 5 \times 5$  wheal. Of the 329 patients with PST positivity, 31 were male (9%) and 298 were female (91%) ( $p < 0.0001$ ). Of the 170 patients that tested positive  $\geq 5 \times 5$  wheal, 48 were male (28%) and 122 were female (72%) ( $p < 0.0001$ ). In total, 139 patients were positive to benzylpenicillin polylysine in the  $\geq 3 \times 3$  wheal group and 55 in the  $\geq 5 \times 5$  wheal group. Fifty-five patients were positive to PCN G in  $\geq 3 \times 3$  wheal group and 65 in the  $\geq 5 \times 5$  wheal group. We compared the differences between solely positive rates of benzylpenicillin polylysine and penicillin G on PST versus all the reagents including major and minor determinants plus amoxicillin. In the  $\geq 3 \times 3$  wheal group benzylpenicillin polylysine and penicillin G showed the following positive results per Cohort: Cohort 1-35%, Cohort 2-52%, Cohort 3-47%, and Cohort 4-32%. Figure 1. The results in the  $\geq 5 \times 5$  wheal group are as follows: Cohort 1-32%, Cohort 2-54%, Cohort 3-47%, and Cohort 4-50%. Figure 2. When using all reagents the percent positive increased as high as 91% in the  $\geq 3 \times 3$  wheal group and 100% in the  $\geq 5 \times 5$  wheal group. Both skin prick testing and intradermal testing was used to determine positive PST. A large majority of our patients tested positive to

intradermal testing. In the  $\geq 3 \times 3$  wheal group, 94% of patients tested positive on intradermal testing ( $p < 0.0001$ ). Of the  $\geq 5 \times 5$  wheal group, 17 (10%) were positive with prick testing and 153 (90%) were positive with intradermal testing ( $p < 0.0001$ ). Ninety percent of patients tested positive with intradermal testing while only 10% tested positive via prick testing ( $p < 0.0001$ ).

Positive results were classified on a yearly basis and can be found in the supplement (Tables E1 and E2). The yearly results were then classified into 5 cohorts using different time frames:

Cohort 1: 2001-2005, Cohort 2: 2006-2010, Cohort 3: 2011-2015, and Cohort 4: 2016-2017.

These results are reflected in Tables 1 and 2.

Cohort 1:

6,536 patients underwent PST from 2001-2005. Of those 6,536 patients, 110 patients had a positive PST (1.7%) using a  $\geq 3 \times 3$  wheal. Of those 110 patients with a positive PST, 54 total patients had benzylpenicillin polylysine positivity. 38 total patients were positive to penicillin G. 39 total patients were positive to penicilloate. 34 patients were positive to amoxicillin.

54 patients (0.8%) had a positive PST using a  $\geq 5 \times 5$  wheal. Of those 54 patients with a positive PST, 19 total patients had benzylpenicillin polylysine positivity, 19 to penicillin G, 15 to penicilloate, and 17 to amoxicillin.

Cohort 2:

10,372 patients underwent PST from 2006-2010. Of those 10,372 patients, 130 patients had a positive PST (1.3%) using a  $\geq 3 \times 3$  wheal. When comparing positive PST rates from Cohort 1 to Cohort 2 there is a change from 1.7% to 1.25% ( $p = 0.03$ ). Of those 130 patients with a positive PST, 52 total patients had benzylpenicillin polylysine positivity. 52 total patients were positive to

penicillin G.36 total patients were positive to penicilloate.33 patients were positive to amoxicillin.

72 patients (0.7%) had a positive PST using a  $\geq 5 \times 5$  wheal. When comparing positive PST rates from Cohort 1 to Cohort 2 there is a change from 0.8% to 0.7% ( $p = 0.762$ ). Of those 72 patients with a positive PST, 19 total patients had benzylpenicillin polylysine positivity..33 total patients were positive to penicillin G.14 total patients were positive to penicilloate.22 patients were positive to amoxicillin.

Cohort 3:

10,640 patients underwent PST from 2011-2015. Of those 10,640 patients, 67 patients had a positive PST (0.6%) using a  $\geq 3 \times 3$  wheal. When comparing positive PST rates from Cohort 1 to Cohort 3 there is a change from 1.7% to 0.6% ( $p < 0.0001$ ) Of those 67 patients with a positive PST, 27 total patients had benzylpenicillin polylysine positivity , 22 to penicillin G, 20 to penicilloate, and 23 to amoxicillin.

34 patients (0.3%) had a positive PST using a  $\geq 5 \times 5$  wheal. When comparing positive PST rates from Cohort 1 to Cohort 3 there is a change from 0.8% to 0.3% ( $p < 0.0001$ ). Of those 34 patients with a positive PST, 13 total patients had benzylpenicillin polylysine positivity, 12 to penicillin G, 12 to penicilloate, and 10 to amoxicillin.

Cohort 4:

3,335 patients underwent PST from 2016-2017. Of those 3,335 patients, 22 patients had a positive PST (0.6%) using a  $\geq 3 \times 3$  wheal. When comparing positive PST rates from Cohort 1 to Cohort 4 there is a change from 1.7% to 0.6% ( $p \text{ value} < 0.0001$ ) Of those 22 patients with a

positive PST, 6 total patients had benzylpenicillin polylysine positivity. 5 total patients were positive to penicillin G. 7 total patients were positive to penicilloate. 8 patients were positive to amoxicillin.

10 patients (0.3%) had a positive PST using a  $\geq 5 \times 5$  wheal. When comparing positive PST rates from Cohort 1 to Cohort 4 there is a change from 0.8% to 0.3% ( $p$  value=0.0034). Of those 10 patients with a positive PST, 4 total patients had benzylpenicillin polylysine positivity, 1 to penicillin G, 3 to penicilloate, and 3 to amoxicillin.

When the positive PST rates for a  $\geq 3 \times 3$  wheal were compared from Cohort 2- 1.25%, Cohort 3- 0.6%, and Cohort 4- 0.6% to Cohort 1-1.7%, there was a significant decrease in positive PST rates ( $p$  0.0278;  $<0.0001$ ;  $<0.0001$ , respectively). Figure 3. Similar results were shown in the  $\geq 5 \times 5$  wheal as positive PST decreased from 0.8% to 0.3% ( $p = 0.003$ ). Figure 4.

In the  $\geq 3 \times 3$  wheal positive PST group, solely penicilloate positivity increased from 8% in Cohort 1 to 27% in Cohort 4 ( $p = 0.04$ ). Figures 5 and 6. The percent positive for each reagent on a yearly basis can be found in the supplement (Figures E1-E8). There was no significant differences in sole benzylpenicillin polylysine positivity in 3mm and 5 mm group, sole amoxicillin positivity in either the 3 mm and 5 mm wheal groups, and no significant difference in the penicilloate data 5mm wheal group.

**Discussion:**

We found in a large population based study that positivity on PST continues to decrease over time. This data supports the work of Macy et al<sup>6</sup> where it was shown that from 1995-2007, PST positive rates had fallen. This is also in concordance with other previous studies conducted in the United States that have shown decreasing PST positivity overtime.<sup>11,13-15</sup> We show that using both a  $\geq 3 \times 3$  (Figure 1) and  $\geq 5 \times 5$  (Figure 2) wheal as determining a positive skin test shows a decrease in skin test positivity overtime. The decrease in clinically confirmed penicillin allergy overtime is likely multifactorial. The patient population in whom are tested also affects the rate of positive testing. For example, if the PST is only used for patients with a convincing history of immediate adverse reaction (IgE-mediated) and higher pre-test probability, the higher the rates of PST positivity compared to testing of more liberal definition of a penicillin allergy. This is supported by the increased likelihood of positivity on PST when tested in the inpatient versus outpatient testing.

In addition, the age at which testing occurs can be important as well. We studied a small number of pediatric patients and showed a relatively high percentage of positive results (3.5%) when compared to our adult population. Another factor possibly contributing to decrease in PST positivity over time is the length of time between testing and original reaction. The greater the time interval from the initial reaction to PST, the less likely a positive result will be recorded.<sup>21-23</sup>

In addition the increase rate of penicillin allergy evaluations secondary to increased antimicrobial stewardship awareness could have also lead to decrease in PST positivity overtime. Previous studies have suggested that utilizing PST with benzylpenicillin polylysine and penicillin G, followed by a 250 mg oral amoxicillin challenge is both safe and effective in determining penicillin tolerance.<sup>11</sup> N.F. Adkinson Jr. et al<sup>12</sup> recently concluded after conducting a meta-

analysis of 50 years of PST that without testing for penicillin minor determinants and amoxicillin that patients with sensitivity to penicillin would be missed using the major determinant and penicillin G alone. In that meta-analysis, only 36-68% percent of patients were positive to the major determinant mixture alone. By adding minor determinant testing, there was an increase in PST positivity by 23-28%. Amoxicillin alone added an increase in positivity by 2-8%. Our data further supports this conclusion by showing a trend of increasing positivity over time to the minor determinants and amoxicillin.

It has been reported in European studies that amoxicillin allergy is increasing both by patient report and on PST.<sup>16</sup> It has been reported that a number of patients that test positive on PST tested positive solely to amoxicillin.<sup>8-10,16</sup> Torres et al<sup>16</sup> showed that amoxicillin positivity on PST was the most common positive component on PST even when co-testing with benzylpenicillin polylysine and minor determinants were used. It has been recently reported by Siew et al, that in the UK amoxicillin monosensitization is common (48%) and that 70% of patients had negative testing to both major and minor determinants.<sup>25</sup> Solensky et al<sup>24</sup> showed that the trend for increasing amoxicillin and minor determinant positivity on PST is also present in the United States. Our data further confirms these findings that the trend for minor determinant and amoxicillin positivity is increasing overtime. We demonstrate that despite benzylpenicillin polylysine solely positive rates remaining relatively stable over time, the minor penicillin determinants and amoxicillin play an important role in PST and their adoption into standard protocol for routine PST should be considered.

The majority of patients that tested positive on PST were female. Of the 30,883 patients tested, 20,658 were female and 10,225 were male. Female patients are more likely to state if they have a penicillin allergy when compared to their male counterparts. While our study shows that female

positivity on PST is much larger than the male population, this could be more from referral bias as females are more likely to have their penicillin allergy documented. Over 90% of patients in the  $\geq 3 \times 3$  wheal group were female. The overall number between female and male positivity showed 91% were female and 9% were male ( $p < 0.0001$ ). Similar results occurred in the  $\geq 5 \times 5$  wheal group. Of the 170 positive patients with a  $\geq 5 \times 5$  wheal, 72% were female and 28% were male ( $p < 0.0001$ ). This confers with previously reported trends that female sex may be a risk factor for penicillin allergy.<sup>8</sup>

One limitation of the study was that this study included retrospective data from a single institution and with referral bias; this may affect the generalizability of our findings. There was no FDA approved product for Pre-Pen between the years of 2004-2010. During that time, our institution made our own Pre-Pen mixture and this may affect the generalizability of our findings as this was a mixture that was not standardized at the time. In our study, we had a large number of outpatient PST when compared to inpatient testing. While we still demonstrated a sizable inpatient PST population, institutions where the majority of PST occurs in the inpatient setting could affect results as well. In the smaller data we had, we demonstrated that inpatient PST resulted in higher positivity and therefore could affect other sites where inpatient PST dominates. Another limitation is that currently there is no FDA approved agent that includes a mixture for minor determinants or amoxicillin to be used for PST. Moreover, as we made our own minor determinant and amoxicillin mixture these results may not be generalizable to mixtures used at other institutions. Hence, with our findings in addition to the recent findings of Solensky et al the need for a FDA standard PST kit with minor determinants and amoxicillin is needed now more than ever to accurately find penicillin allergy on skin testing.

Conclusion:

Our study shows that the positive rate of PST continues to decrease with time. This is confirmed in both the  $\geq 3 \times 3$  and  $\geq 5 \times 5$  wheal groups. Positivity to intradermal testing is much larger than by prick testing. Over 90% of patients were positive on intradermal testing alone. Females are more likely to have a positive PST than male. Benzylpenicillin polylysine and penicillin G should not be the only determinants tested on PST. If the other minor determinants and amoxicillin are left out from PST, then a large majority of patients with IgE-mediated positive penicillin allergy could be missed.

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**Figure Legend:**

Table 1:  $\geq 3 \times 3$  wheal PST results for Cohorts 1-4

Table 2:  $\geq 5 \times 5$  wheal PST results for Cohorts 1-4

Figure 1: Comparison of Solely Pre-Pen and PCN G vs All Reagents by Cohort in  $\geq 3 \times 3$  Wheal Group

Figure 2: Comparison of Solely Pre-Pen and PCN G vs All Reagents by Cohort in  $\geq 5 \times 5$  Wheal Group

Figure 3: PST Percent Positive  $\geq 3 \times 3$  Wheal

Figure 4: PST Percent Positive  $\geq 5 \times 5$  Wheal

Figure 5: Cohort Percent of Each Solely Positive PST Component in  $\geq 3 \times 3$  Wheal Group

Figure 6: Cohort Percent of Each Solely Positive PST Component in  $\geq 5 \times 5$  Wheal Group

**Table 1:  $\geq 3 \times 3$  wheal PST results for Cohorts 1-4:**

| Cohort        | # pts tested | Total number of positive PST patient results | Total Pre-Pen | Solely Pre-Pen | Total PCN G | Solely PCN G | Total Penicilloate | Solely Penicilloate | Total Amoxicillin | Solely Amoxicillin |
|---------------|--------------|----------------------------------------------|---------------|----------------|-------------|--------------|--------------------|---------------------|-------------------|--------------------|
| 1 (2001-2005) | 6536         | 110 (1.7%)                                   | 54 (49%)      | 30 (27%)       | 38 (35%)    | 12 (11%)     | 39 (35%)           | 9 (8%)              | 34 (31%)          | 13 (12%)           |
| 2 (2006-2010) | 10372        | 130 (1.3%)                                   | 52 (40%)      | 27 (21%)       | 52 (40%)    | 49 (31%)     | 36 (28%)           | 20 (15%)            | 33 (25%)          | 28 (22%)           |
| 3 (2011-2015) | 10640        | 67 (0.6%)                                    | 27 (40%)      | 23 (34%)       | 22 (33%)    | 9 (13%)      | 29 (30%)           | 7 (15%)             | 23 (34%)          | 11 (16%)           |
| 4 (2016-2017) | 3335         | 22 (0.6%)                                    | 6 (27%)       | 4 (18%)        | 5 (23%)     | 3 (14%)      | 7 (32%)            | 6 (27%)             | 8 (36%)           | 7 (32%)            |

**Table 2:  $\geq 5 \times 5$  wheal PST results for Cohorts 1-4:**

| Cohort        | # pts tested | Total number of positive PST patient results | Total Pre-Pen | Solely Pre-Pen | Total PCN G | Solely PCN G | Total Penicilloate | Solely Penicilloate | Total Amoxicillin | Solely Amoxicillin |
|---------------|--------------|----------------------------------------------|---------------|----------------|-------------|--------------|--------------------|---------------------|-------------------|--------------------|
| 1 (2001-2005) | 6536         | 54 (0.8%)                                    | 19 (35%)      | 10 (19%)       | 19 (35%)    | 7 (13%)      | 15 (28%)           | 7 (13%)             | 17 (31%)          | 12 (22%)           |
| 2 (2006-2010) | 10372        | 72 (0.7%)                                    | 19 (26%)      | 16 (22%)       | 33 (46%)    | 23 (32%)     | 14 (19%)           | 7 (10%)             | 22 (31%)          | 14 (19%)           |
| 3 (2011-2015) | 10640        | 34 (0.3%)                                    | 13 (38%)      | 11 (32%)       | 12 (35%)    | 5 (15%)      | 12 (35%)           | 5 (10%)             | 10 (29%)          | 5 (15%)            |
| 4 (2016-2017) | 3335         | 10 (0.3%)                                    | 4 (40%)       | 4 (40%)        | 1 (10%)     | 1 (10%)      | 3 (30%)            | 3 (30%)             | 3 (30%)           | 2 (20%)            |

**Figure 1: Comparison of Solely Pre-Pen and PCN G vs All Reagents by Cohort in  $\geq 3 \times 3$  Wheal Group**

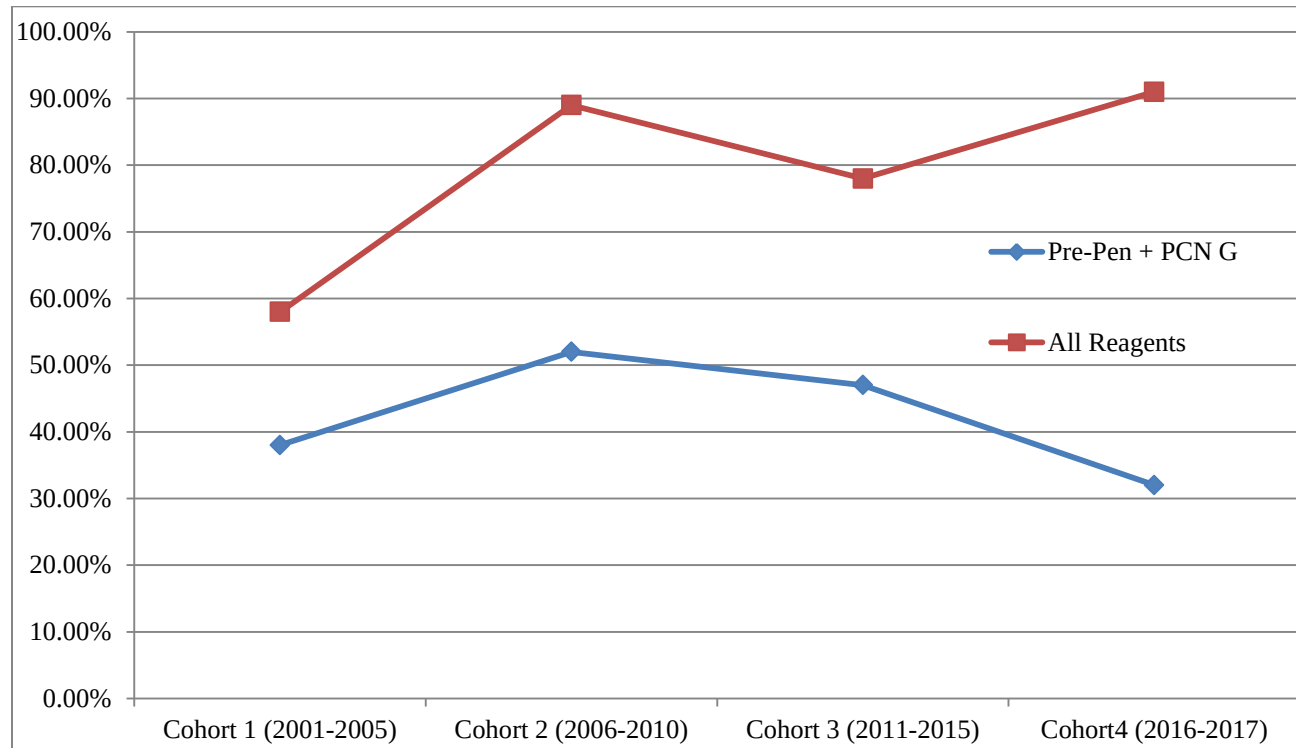


**Figure 2: Comparison of Solely Pre-Pen and PCN G vs All Reagents by Cohort in  $\geq 5 \times 5$  Wheal Group**

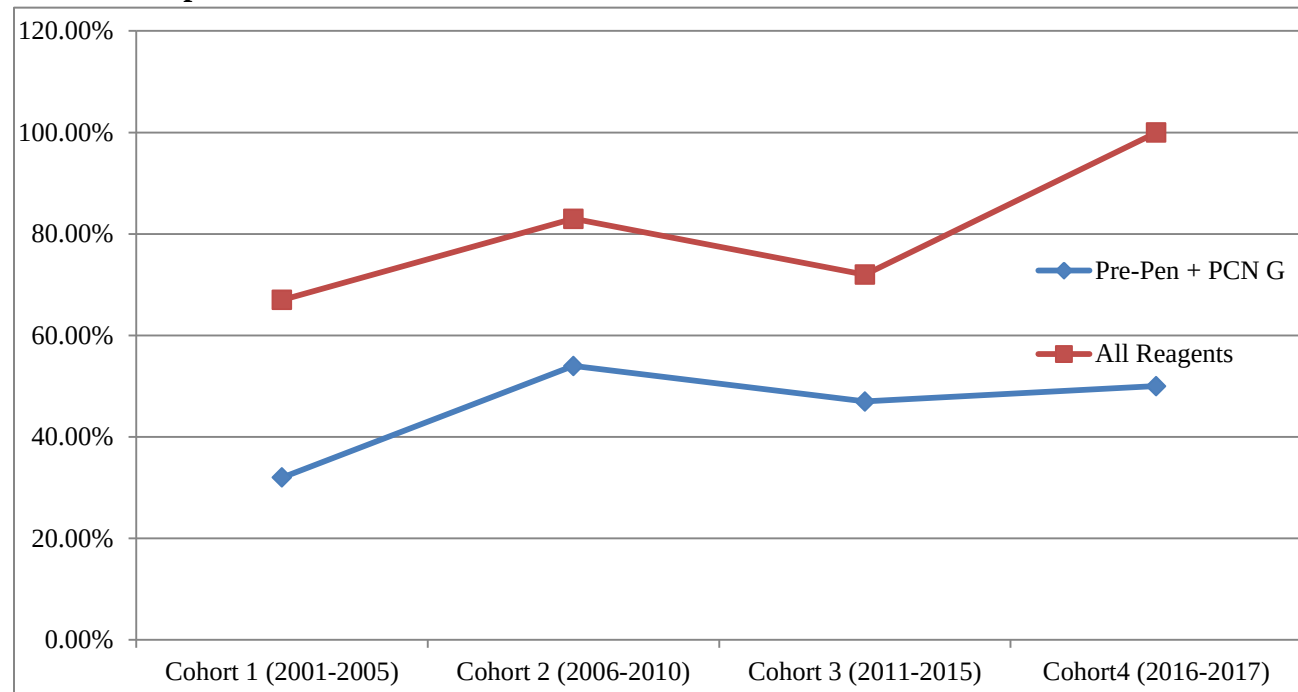


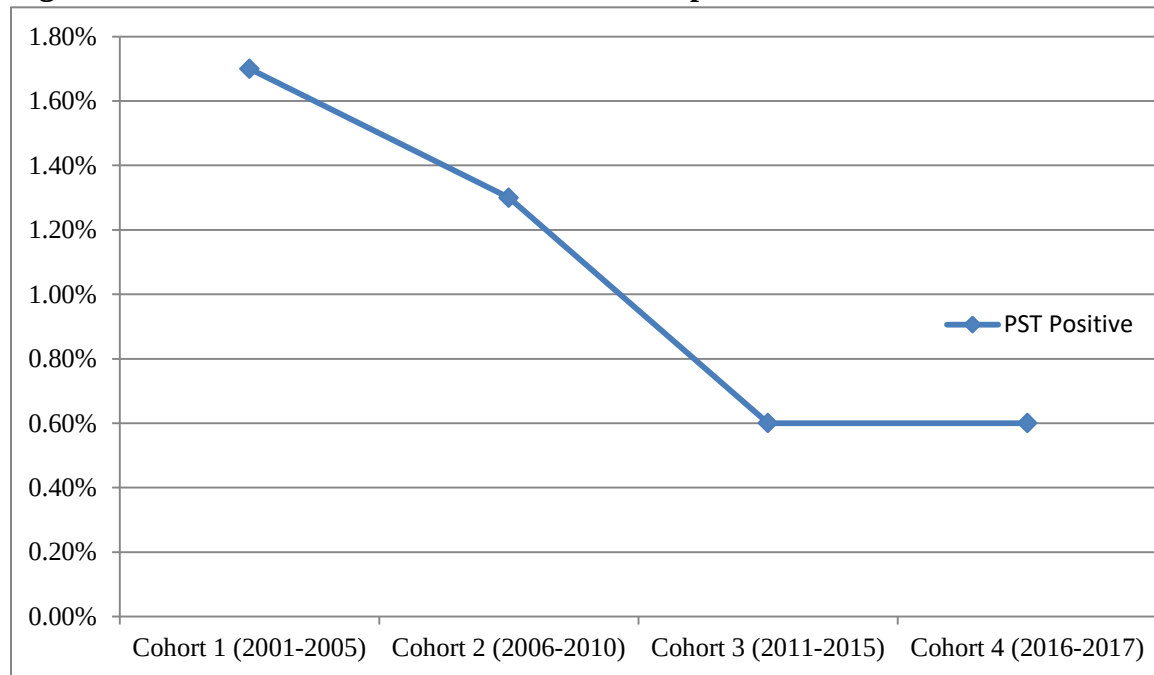
**Figure 3: PST Percent Positive  $\geq 3 \times 3$  Wheal Group**

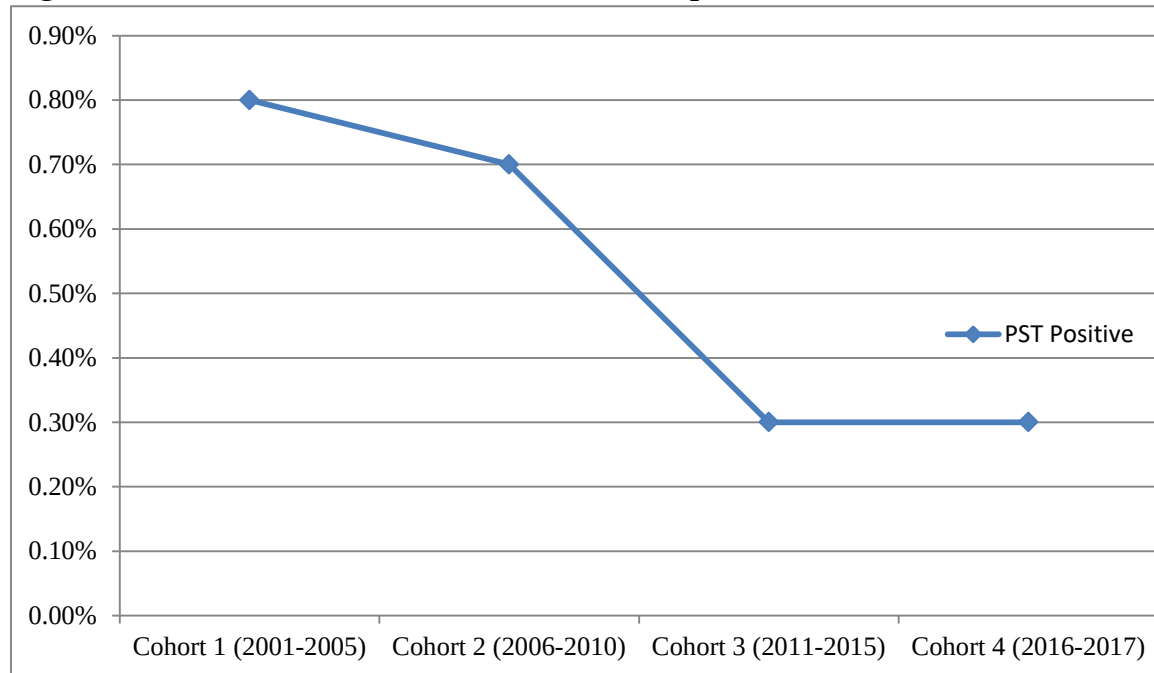
**Figure 4: PST Percent Positive  $\geq 5 \times 5$  Wheal Group**

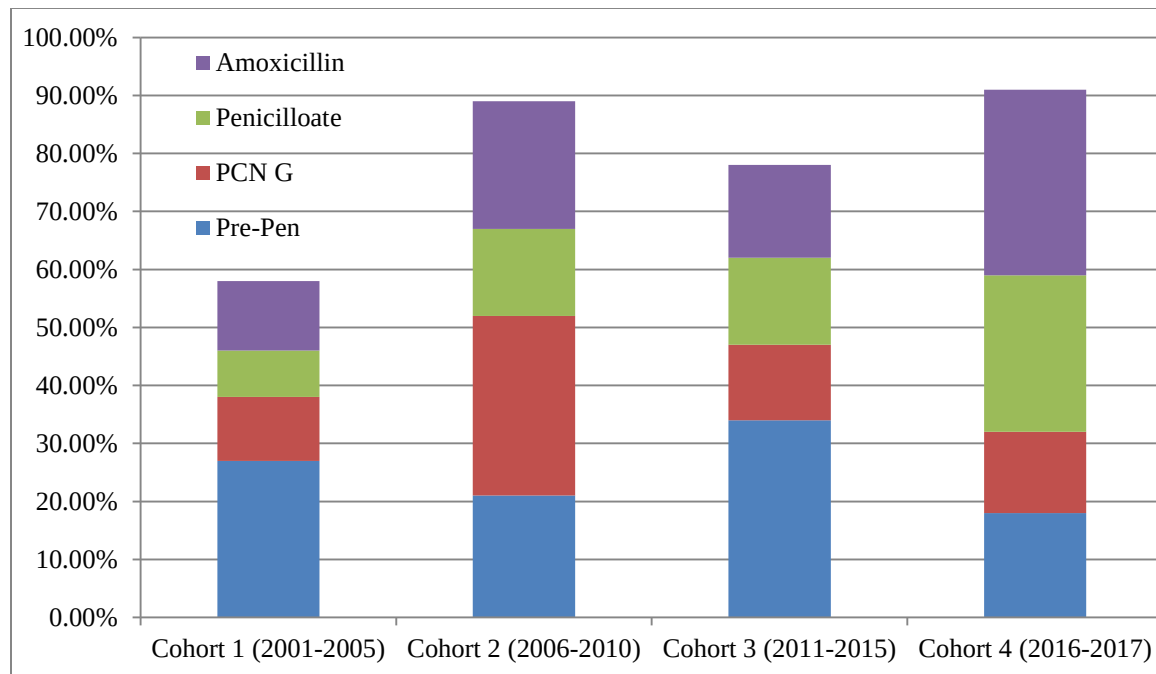
**Figure 5: Cohort Percent of Each Solely Positive PST Component in  $\geq 3 \times 3$  Wheal Group**

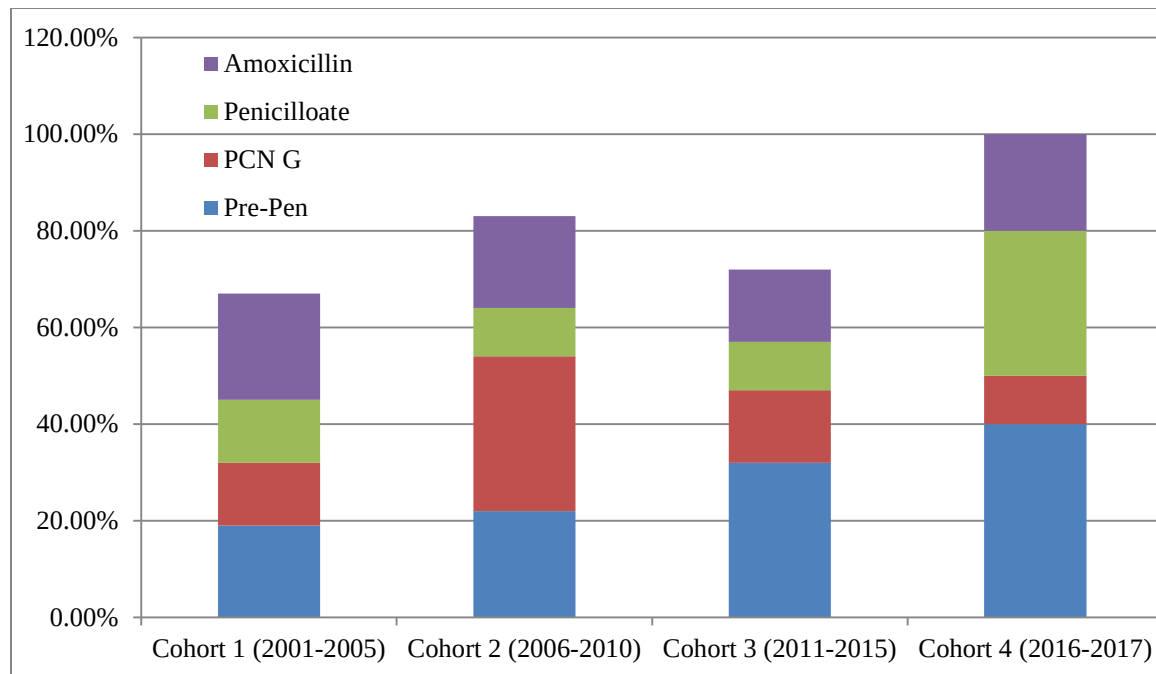
**Figure 6: Cohort Percent of Each Solely Positive PST Component in  $\geq 5 \times 5$  Wheal Group**

**Figure E1: Yearly Percent of Each Solely Positive PST Component in  $\geq 3 \times 3$  Wheal Group from 2001-2005**

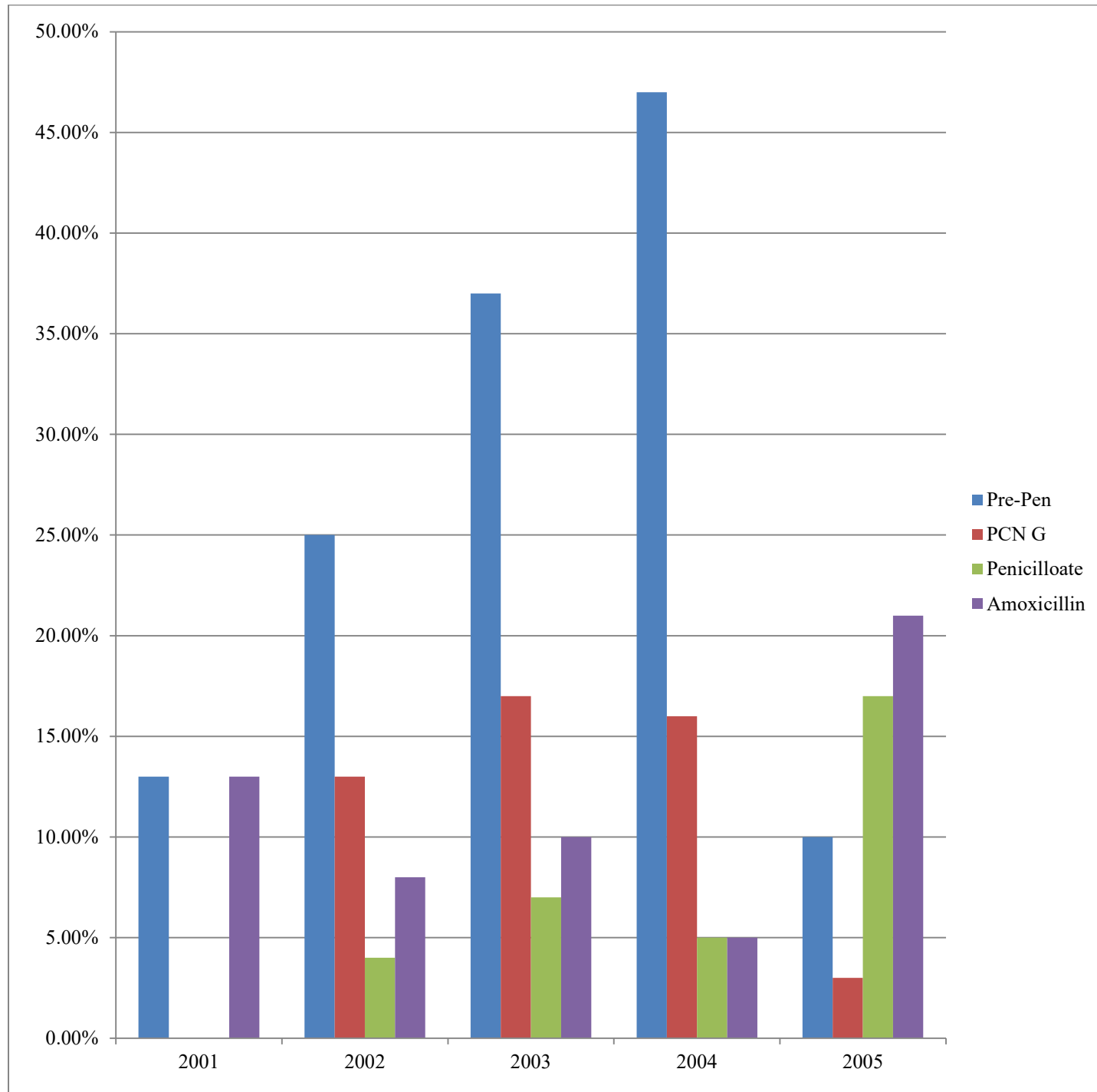


**Figure E2: Yearly Percent of Each Solely Positive PST Component in  $\geq 3 \times 3$  Wheal Group from 2006-2010**

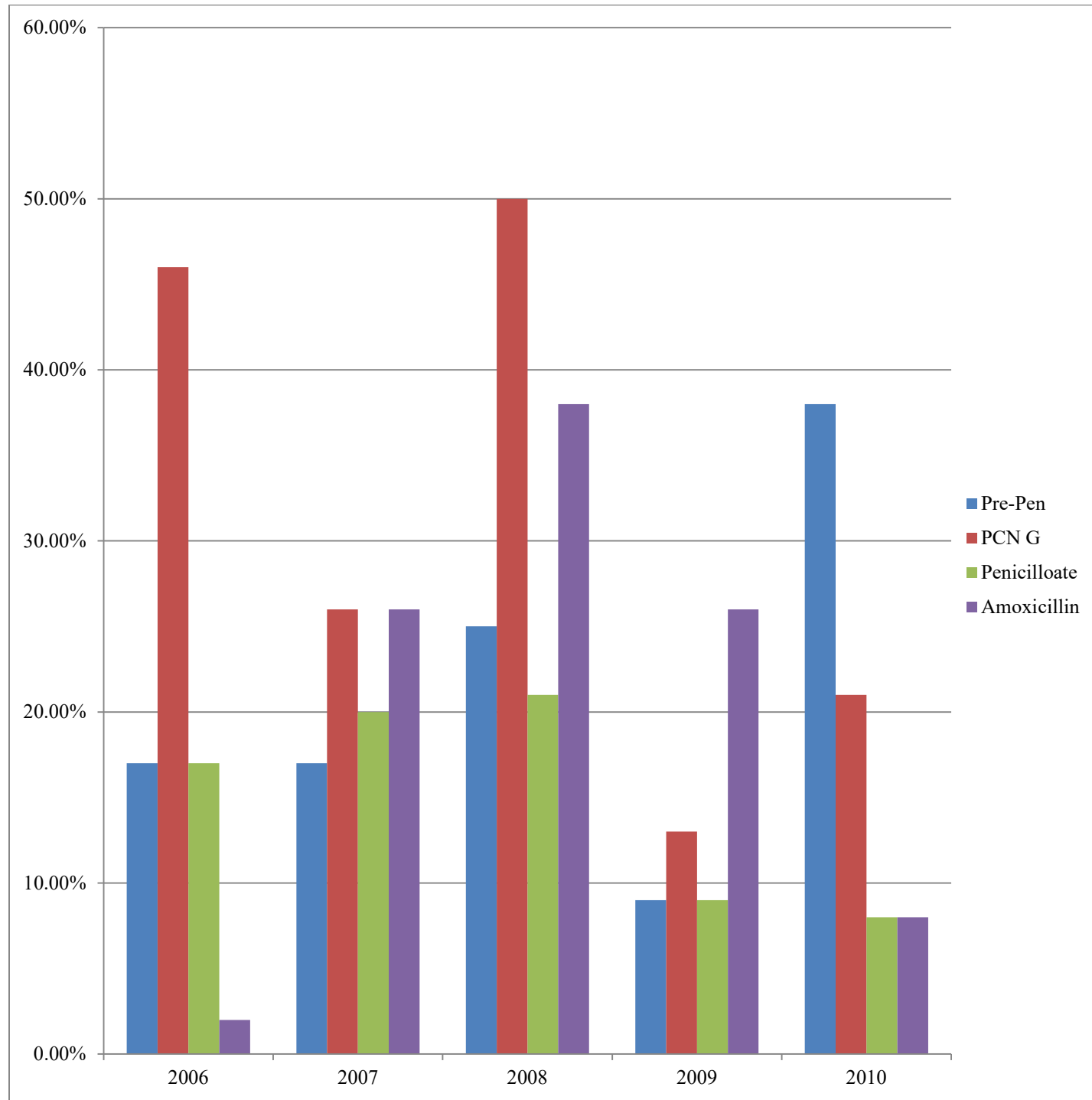


**Figure E3: Yearly Percent of Each Solely Positive PST Component in  $\geq 3 \times 3$  Wheal Group from 2011-2015**

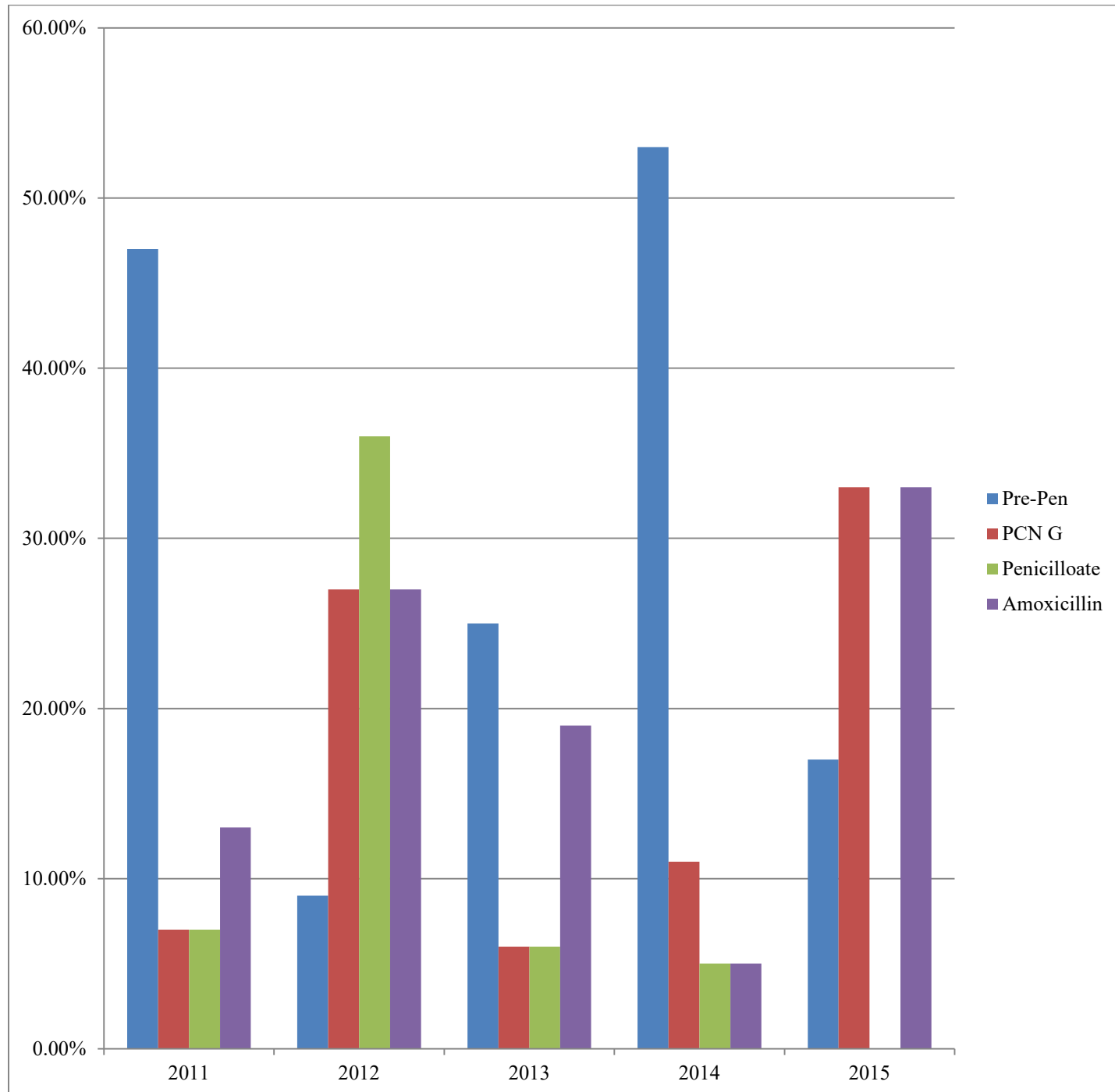


**Figure E4: Yearly Percent of Each Solely Positive PST Component in  $\geq 3 \times 3$  Wheal Group from 2016-2017**

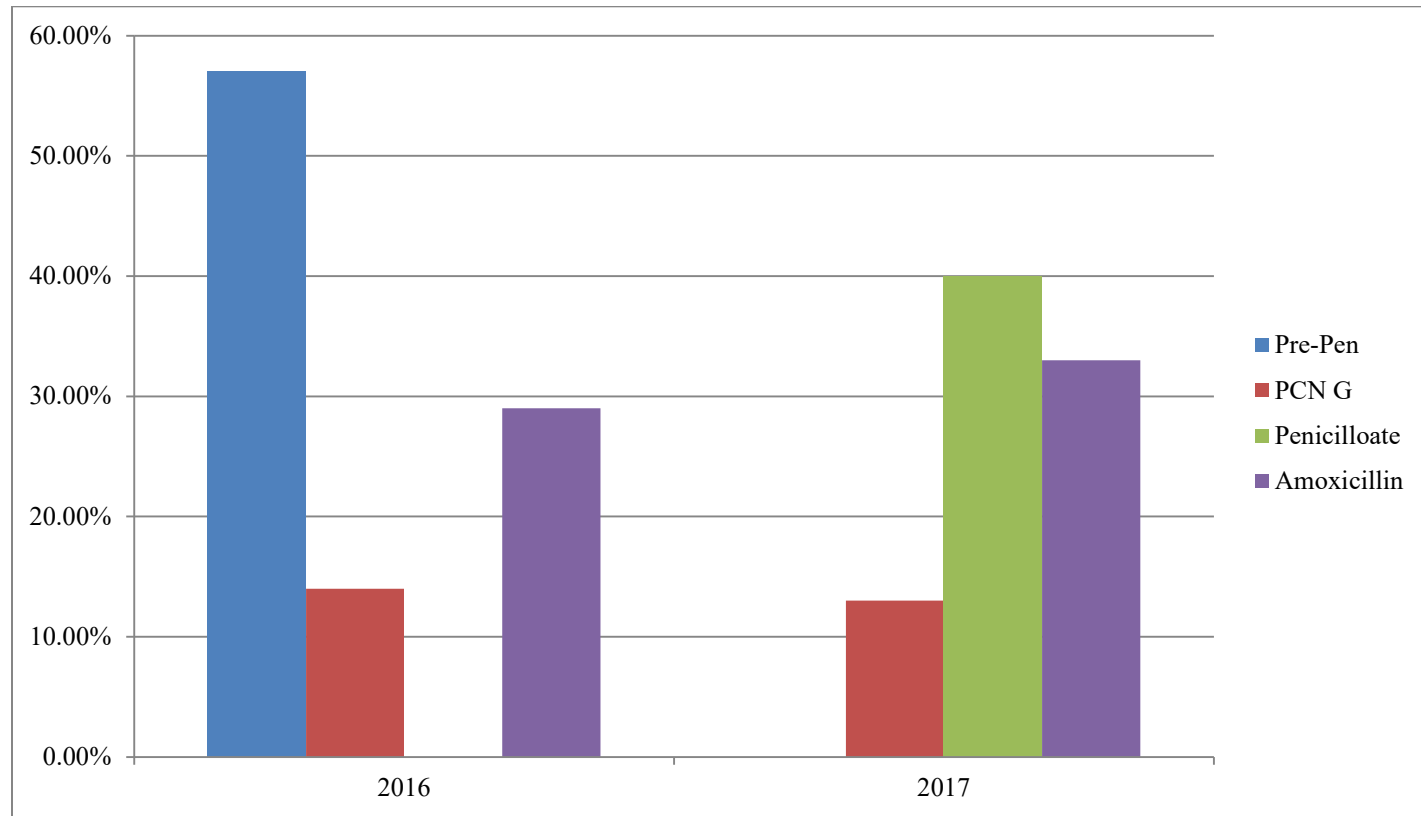


**Figure E5: Yearly Percent of Each Solely Positive PST Component in  $\geq 5 \times 5$  Wheal Group from 2001-2005**

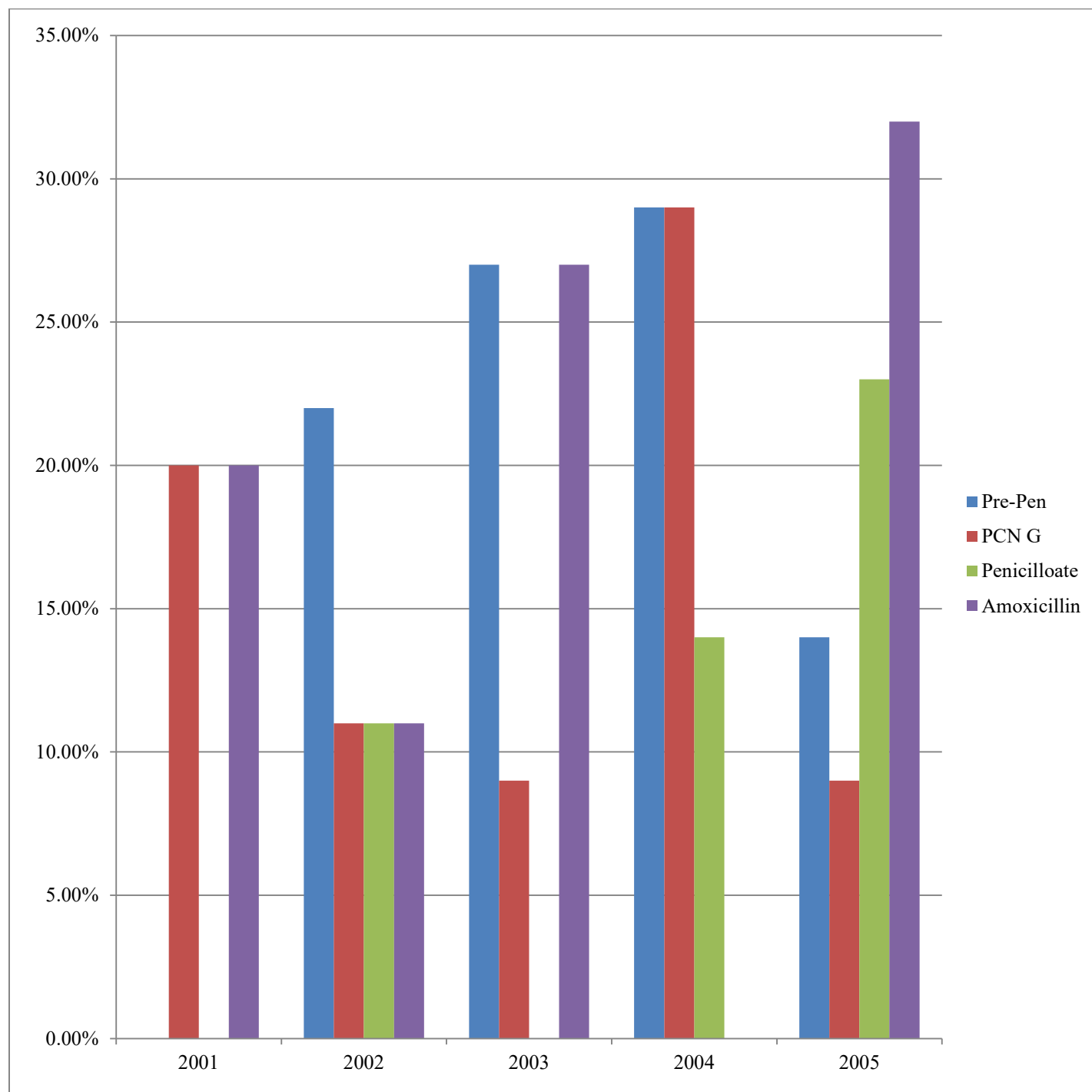


**Figure E6: Yearly Percent of Each Solely Positive PST Component in  $\geq 5 \times 5$  Wheal Group from 2006-2010**

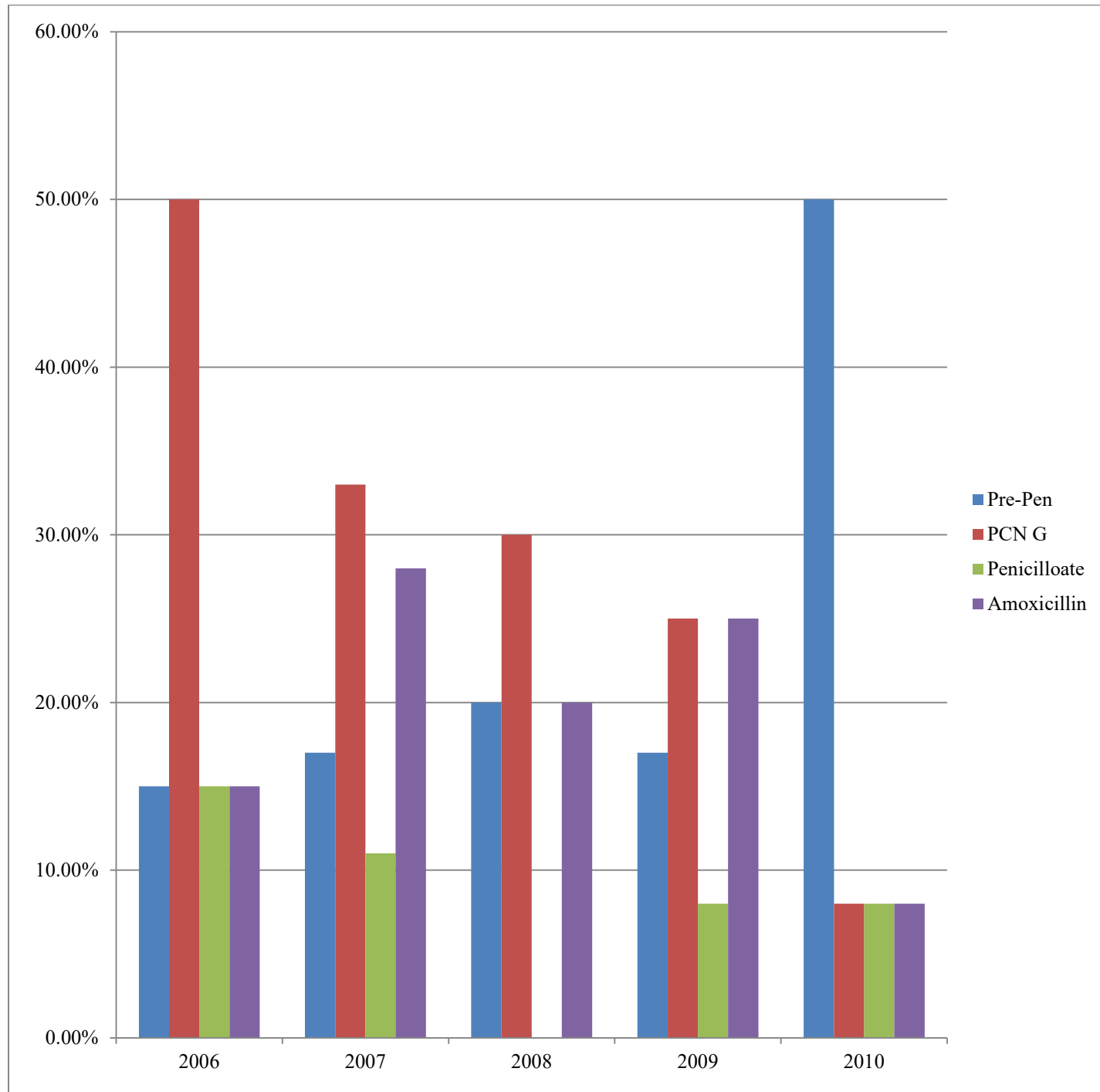


**Figure E7: Yearly Percent of Each Solely Positive PST Component in  $\geq 5 \times 5$  Wheal Group from 2011-2015**

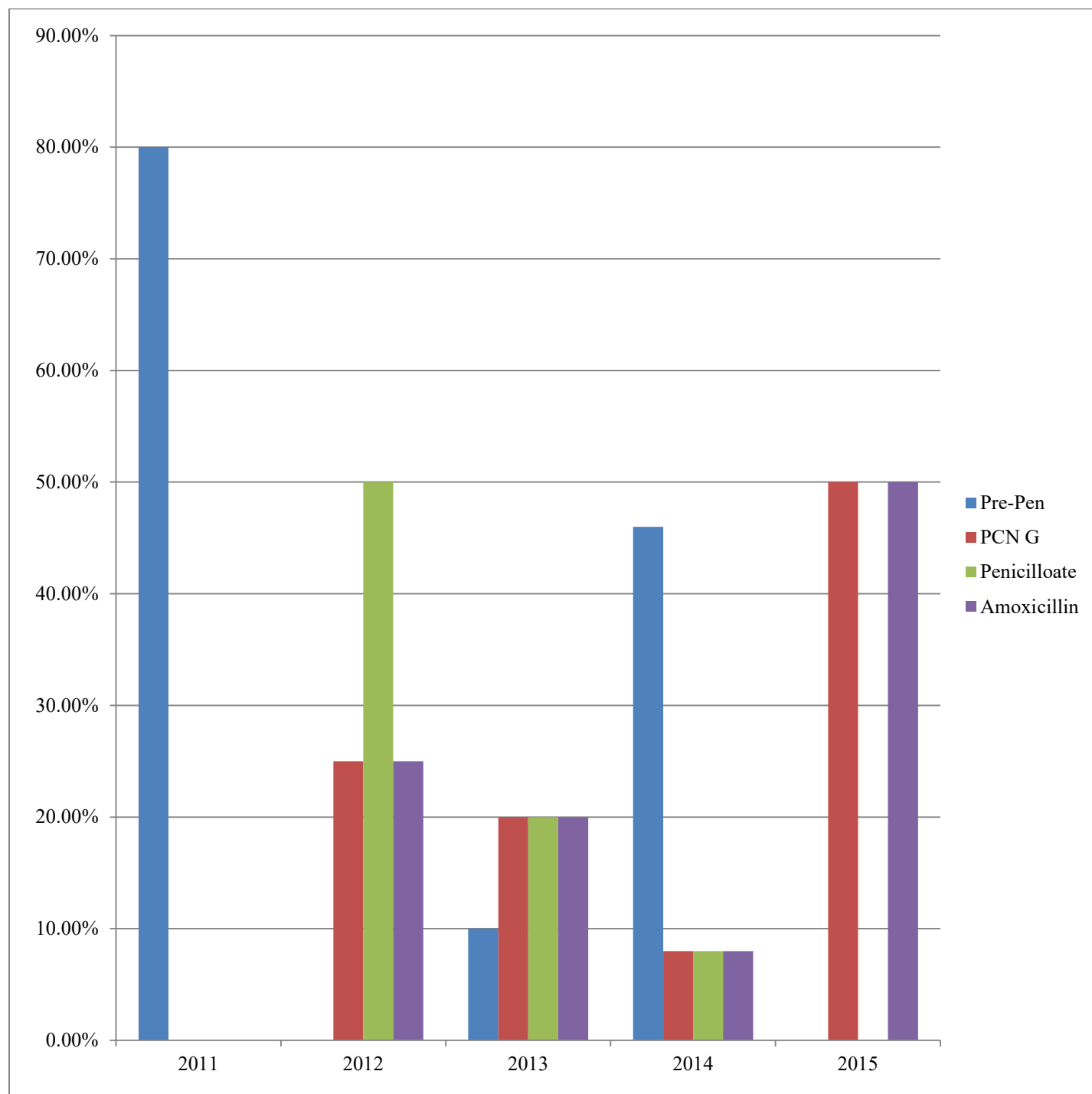
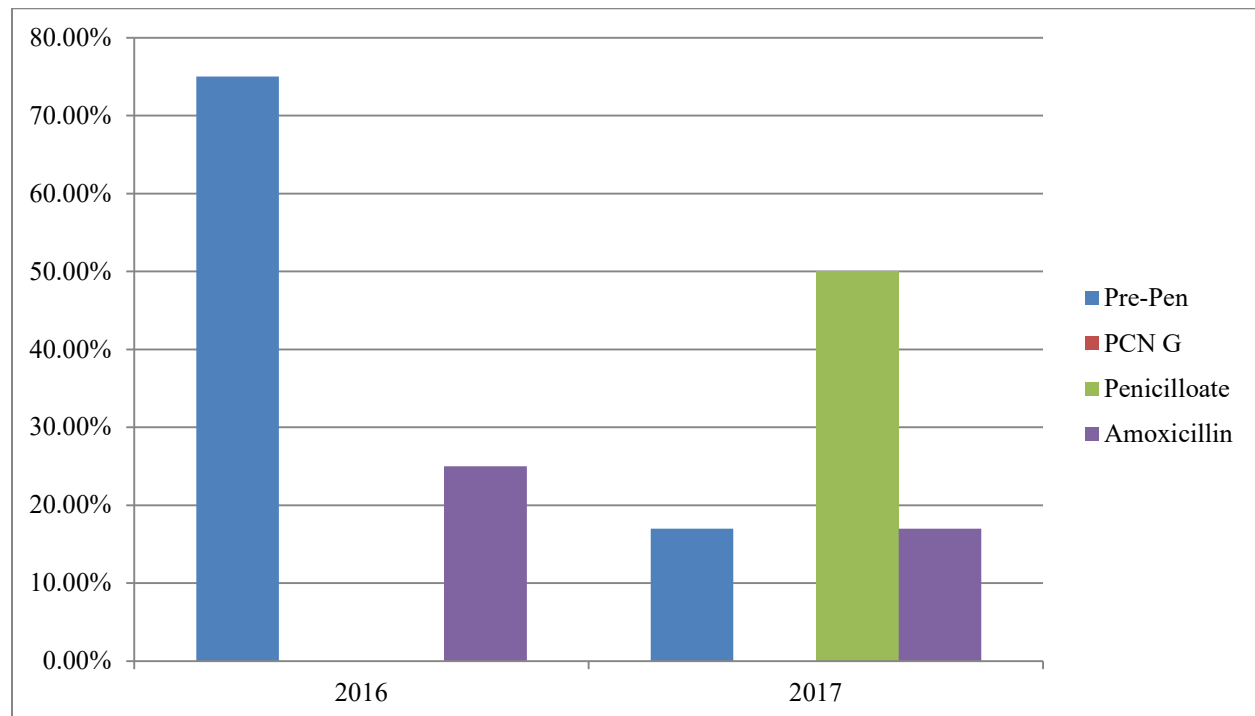


**Figure E8: Yearly Percent of Each Solely Positive PST Component in  $\geq 5 \times 5$  Wheal Group from 2016-2017**



**Supplemental Figure Legend:**

Table E1: Yearly PST results for  $\geq 3 \times 3$  wheal positivity

Table E2: Yearly PST results for  $\geq 5 \times 5$  wheal positivity

Figure E1: Yearly Percent of Each Solely Positive PST Component in  $\geq 3 \times 3$  Wheal Group from 2001-2005

Figure E2: Yearly Percent of Each Solely Positive PST Component in  $\geq 3 \times 3$  Wheal Group from 2006-2010

Figure E3: Yearly Percent of Each Solely Positive PST Component in  $\geq 3 \times 3$  Wheal Group from 2011-2015

Figure E4: Yearly Percent of Each Solely Positive PST Component in  $\geq 3 \times 3$  Wheal Group from 2016-2017

Figure E5: Yearly Percent of Each Solely Positive PST Component in  $\geq 5 \times 5$  Wheal Group from 2001-2005

Figure E6: Yearly Percent of Each Solely Positive PST Component in  $\geq 5 \times 5$  Wheal Group from 2006-2010

Figure E7: Yearly Percent of Each Solely Positive PST Component in  $\geq 5 \times 5$  Wheal Group from 2011-2015

Figure E8: Yearly Percent of Each Solely Positive PST Component in  $\geq 5 \times 5$  Wheal Group from 2016-2017

**Table E1: Yearly PST results for  $\geq 3 \times 3$  wheal positivity**

| Year         | # pts tested | Total number of positive PST patient results | Total Pre-Pen    | Solely Pre-Pen  | Total PCN G      | Solely PCN G    | Total Penicilloate | Solely Penicilloate | Total Amoxicillin | Solely Amoxicillin |
|--------------|--------------|----------------------------------------------|------------------|-----------------|------------------|-----------------|--------------------|---------------------|-------------------|--------------------|
| 2001         | 652          | 8 (1.2%)                                     | 5 (63%)          | 1 (13%)         | 4 (50%)          | 0 (0%)          | 3 (38%)            | 0 (0%)              | 3 (38%)           | 1 (13%)            |
| 2002         | 1149         | 24 (2.1%)                                    | 16 (67%)         | 6 (25%)         | 10 (42%)         | 3 (13%)         | 7 (29%)            | 1 (4%)              | 7 (29%)           | 2 (8%)             |
| 2003         | 1596         | 30 (1.8%)                                    | 17 (57%)         | 11 (37%)        | 8 (27%)          | 5 (17%)         | 9 (30%)            | 2 (7%)              | 8 (27%)           | 3 (10%)            |
| 2004         | 1558         | 19 (1.2%)                                    | 11 (58%)         | 9 (47%)         | 6 (32%)          | 3 (16%)         | 4 (21%)            | 1 (5%)              | 3 (16%)           | 1 (5%)             |
| 2005         | 1581         | 29 (1.8%)                                    | 5 (17%)          | 3 (10%)         | 10 (34%)         | 1 (3%)          | 16 (55%)           | 5 (17%)             | 13 (45%)          | 6 (21%)            |
| 2006         | 1781         | 24 (1.3%)                                    | 5 (21%)          | 4 (17%)         | 15 (63%)         | 11 (46%)        | 4 (17%)            | 4 (17%)             | 3 (13%)           | 2 (8%)             |
| 2007         | 1919         | 35 (1.8%)                                    | 6 (17%)          | 6 (17%)         | 12 (34%)         | 9 (26%)         | 11 (31%)           | 7 (20%)             | 11 (31%)          | 9 (26%)            |
| 2008         | 2079         | 24 (1.1%)                                    | 24 (100%)        | 6 (25%)         | 4 (17%)          | 12 (50%)        | 7 (29%)            | 5 (21%)             | 2 (8%)            | 9 (38%)            |
| 2009         | 2218         | 23 (1.0%)                                    | 6 (26%)          | 2 (9%)          | 12 (52%)         | 3 (13%)         | 10 (43%)           | 2 (9%)              | 11 (48%)          | 6 (26%)            |
| 2010         | 2375         | 24 (1.0%)                                    | 11 (46%)         | 9 (38%)         | 9 (38%)          | 5 (21%)         | 4 (17%)            | 2 (8%)              | 6 (25%)           | 2 (8%)             |
| 2011         | 2512         | 15 (0.6%)                                    | 8 (53%)          | 7 (47%)         | 4 (27%)          | 1 (7%)          | 5 (33%)            | 1 (7%)              | 3 (20%)           | 2 (13%)            |
| 2012         | 2307         | 11 (0.5%)                                    | 1 (9%)           | 1 (9%)          | 3 (27%)          | 3 (27%)         | 4 (36%)            | 4 (36%)             | 3 (27%)           | 3 (27%)            |
| 2013         | 1987         | 16 (0.8%)                                    | 5 (31%)          | 4 (25%)         | 5 (31%)          | 1 (6%)          | 7 (44%)            | 1 (6%)              | 9 (56%)           | 3 (19%)            |
| 2014         | 1912         | 19 (0.9%)                                    | 12 (63%)         | 10 (53%)        | 7 (37%)          | 2 (11%)         | 4 (21%)            | 1 (5%)              | 5 (26%)           | 1 (5%)             |
| 2015         | 1922         | 6 (0.3%)                                     | 1 (17%)          | 1 (17%)         | 3 (50%)          | 2 (33%)         | 0 (0%)             | 0 (0%)              | 3 (50%)           | 2 (33%)            |
| 2016         | 1856         | 7 (0.3%)                                     | 4 (57%)          | 4 (57%)         | 1 (14%)          | 1 (14%)         | 0 (0%)             | 0 (0%)              | 2 (29%)           | 2 (29%)            |
| 2017         | 1479         | 15 (1.0%)                                    | 2 (13%)          | 0 (0%)          | 4 (27%)          | 2 (13%)         | 7 (47%)            | 6 (40%)             | 6 (40%)           | 5 (33%)            |
| <b>Total</b> | <b>30883</b> | <b>329 (1.0%)</b>                            | <b>139 (42%)</b> | <b>84 (26%)</b> | <b>117 (36%)</b> | <b>64 (19%)</b> | <b>102 (31%)</b>   | <b>42 (13%)</b>     | <b>98 (30%)</b>   | <b>59 (18%)</b>    |

**Table E2: Yearly PST results for  $\geq 5 \times 5$  wheal positivity:**

| Year         | # pts tested | Total number of positive PST patient results | Total Pre-Pen   | Solely Pre-Pen  | Total PCN G     | Solely PCN G    | Total Penicilloate | Solely Penicilloate | Total Amoxicillin | Solely Amoxicillin |
|--------------|--------------|----------------------------------------------|-----------------|-----------------|-----------------|-----------------|--------------------|---------------------|-------------------|--------------------|
| 2001         | 652          | 5 (0.7%)                                     | 2 (40%)         | 0 (0%)          | 2 (40%)         | 1 (20%)         | 2 (40%)            | 0 (0%)              | 2 (40%)           | 1 (20%)            |
| 2002         | 1149         | 9 (0.7%)                                     | 5 (56%)         | 2 (22%)         | 5 (56%)         | 1 (11%)         | 1 (11%)            | 1 (11%)             | 2 (22%)           | 1 (11%)            |
| 2003         | 1596         | 11 (0.7%)                                    | 5 (45%)         | 3 (27%)         | 2 (18%)         | 1 (9%)          | 2 (18%)            | 0 (0.0%)            | 4 (36%)           | 3 (27%)            |
| 2004         | 1558         | 7 (0.4%)                                     | 3 (43%)         | 2 (29%)         | 3 (43%)         | 2 (29%)         | 2 (29%)            | 1 (14%)             | 1 (14%)           | 0 (0%)             |
| 2005         | 1581         | 22 (1.3%)                                    | 4 (18%)         | 3 (14%)         | 7 (32%)         | 2 (9%)          | 8 (36%)            | 5 (23%)             | 8 (36%)           | 7 (32%)            |
| 2006         | 1781         | 20 (1.1%)                                    | 3 (15%)         | 3 (15%)         | 11 (55%)        | 10 (50%)        | 4 (20%)            | 3 (15%)             | 3 (15%)           | 3 (15%)            |
| 2007         | 1919         | 18 (0.9%)                                    | 3 (17%)         | 3 (17%)         | 8 (44%)         | 6 (33%)         | 3 (17%)            | 2 (11%)             | 6 (33%)           | 5 (28%)            |
| 2008         | 2079         | 10 (0.5%)                                    | 3 (30%)         | 2 (20%)         | 6 (60%)         | 3 (30%)         | 2 (20%)            | 0 (0%)              | 4 (40%)           | 2 (20%)            |
| 2009         | 2218         | 12 (0.5%)                                    | 3 (25%)         | 2 (17%)         | 5 (42%)         | 3 (25%)         | 3 (25%)            | 1 (8%)              | 6 (50%)           | 3 (25%)            |
| 2010         | 2375         | 12 (0.5%)                                    | 7 (58%)         | 6 (50%)         | 3 (25%)         | 1 (8%)          | 2 (17%)            | 1 (8%)              | 3 (25%)           | 1 (8%)             |
| 2011         | 2512         | 5 (0.2%)                                     | 4 (80%)         | 4 (80%)         | 1 (20%)         | 0 (0%)          | 1 (20%)            | 0 (0%)              | 0 (0%)            | 0 (0%)             |
| 2012         | 2307         | 4 (0.2%)                                     | 0 (0%)          | 0 (0%)          | 1 (25%)         | 1 (25%)         | 2 (50%)            | 2 (50%)             | 1 (25%)           | 1 (25%)            |
| 2013         | 1987         | 10 (0.5%)                                    | 2 (20%)         | 1 (10%)         | 4 (40%)         | 2 (20%)         | 5 (50%)            | 2 (20%)             | 5 (50%)           | 2 (20%)            |
| 2014         | 1912         | 13 (0.6%)                                    | 7 (54%)         | 6 (46%)         | 5 (38%)         | 1 (8%)          | 4 (31%)            | 1 (8%)              | 3 (23%)           | 1 (8%)             |
| 2015         | 1922         | 2 (0.1%)                                     | 0 (0%)          | 0 (0%)          | 1 (50%)         | 1 (50%)         | 0 (0%)             | 0 (0%)              | 1 (50%)           | 1 (50%)            |
| 2016         | 1856         | 4 (0.2%)                                     | 3 (75%)         | 3 (75%)         | 0 (0%)          | 0 (0%)          | 0 (0%)             | 0 (0%)              | 1 (25%)           | 1 (25%)            |
| 2017         | 1479         | 6 (0.4%)                                     | 1 (17%)         | 1 (17%)         | 1 (17%)         | 0 (0%)          | 3 (50%)            | 3 (50%)             | 2 (33%)           | 1 (17%)            |
| <b>Total</b> | <b>30883</b> | <b>170 (0.5%)</b>                            | <b>55 (32%)</b> | <b>41 (24%)</b> | <b>65 (38%)</b> | <b>35 (21%)</b> | <b>44 (26%)</b>    | <b>22 (13%)</b>     | <b>52 (31%)</b>   | <b>33 (19%)</b>    |